ELSEVIER

Pharmacology & Therapeutics 93 (2002) 51–75

Pharmacology & Therapeutics

The diverse molecular mechanisms responsible for the actions of opioids on the cardiovascular system

Michael K. Pugsley*

Department of Pharmacology and Toxicology, XOMA (US) LLC, 2910 Seventh Street, Berkeley, CA 94710, USA

Abstract

The actions of opioid agonist and antagonist drugs have not been well characterized in the heart and cardiovascular system. This stems from the limited role opioid receptors have been perceived to have in the regulation of the cardiovascular system. Instead, the focus of opioid receptor research, for many years, relates to the characterization of the actions of opioid drugs in analgesia associated with receptor activation in the CNS. However, recent studies suggest that opioid receptors have a role in the heart and cardiovascular system. While some of these actions may be mediated by activation of peripheral opioid receptors, others are not, and may result from direct or receptor-independent actions on cardiac tissue and the peripheral vascular system. This review will outline some of the diverse molecular mechanisms that may be responsible for the cardiovascular actions of opioids, and will characterize the role opioid receptors have in several cardiovascular pathophysiological disease states, including hypertension, heart failure, and ischaemic arrhythmogenesis. In many instances, it would appear that the effects of opioid agonists (and antagonists) in cardiovascular disease models may be mediated by opioid receptor-independent actions of these drugs.

Keywords: Opioid; Cardiac arrhythmias; Hypertension; Ischaemic preconditioning; Arylacetamide; Review

Abbreviations: APD, action potential duration; AVP, arginine vasopressin; BNTX, 7-benzylidenenaltrexone; cDNA, complementary DNA; CHF, congestive heart failure; EKC, ethylketocyclazocine; EOP, endogenous opioid peptide; HERG, human ether-a-go-go-related gene; HIF, hypoxia-inducible factor; iNOS, inducible nitric oxide synthase; K_{ATP}, ATP-dependent K^+; MERF, Met^5-enkephalin-Arg^6-Phe^7; MR2266, (−)-5,9α-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan hydrochloride; NF-κB, nuclear factor-κB; NO, nitric oxide; nor-BNI, norbinaltorphimine; NOS, nitric oxide synthase; ODN, oligodeoxynucleotide; PC, preconditioning; PCR, polymerase chain reaction; PKA; protein kinase A; PKC, protein kinase C; POMC, pro-opiomelanocortin; TAN-67; 2-methyl-4aα-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12aα-octahydroquinolino[2,3,3-g]isoquinoline chloride; U-50,488H, trans-(±)-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methane sulphonate; U-62,066E, (5α,7α,8β)-(±)-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]-dec-8-yl]-4-benzeneacetamide hydrochoride, spiradoline; U-69,593; [^{3}H](5α,7α,8β)-(+)-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]-dec-8-yl]-4-benzene-acetamide methane sulphonate; VF, ventricular fibrillation; VT, ventricular tachycardia

Contents

* Tel.: 510-644-1170; fax: 510-841-7805.
E-mail address: pugsley@xoma.com (M.K. Pugsley).

0163-7258/02/$ – see front matter
PII: S0163-7258(02)00165-1

The identification of both agonist and antagonist drugs acting upon the various opioid receptors located in the central and peripheral nervous systems has resulted from the large monetary incentive and market potential for analgesics. Thus, the pharmaceutical industry strives to find new, less toxic analgesics, and in doing so, has created many novel classes of agonist and antagonist drugs with potent analgesic properties, but that retain many undesired side effects. When this increase in development of drugs is combined with rapidly improving molecular techniques used to both distinguish opioid binding sites in native tissue and characterize opioid receptors using heterologous expression systems, an understanding of opioid and non-opioid analgesic mechanism(s) of action may be expected to be resolved. To date, the majority of the studies conducted with opioids have been in neuronal tissue, in an attempt to elucidate the analgesic mechanism of action of these agents. The pharmacological profile of action of most opioid agents on the heart and cardiovascular system to a large degree remains uncharacterized. This review will provide, for the first time, an overview of the complexity of the opioid receptor-dependent and opioid receptor-independent-mediated pharmacological actions of opioids in the cardiovascular system.

1. Introduction: a general overview of opioids

Opium is a poppy extract derived from the plant *Papaver somniferum* that, for many centuries, had been used to produce analgesia and to alleviate pain.

Opium contains several important alkaloid constituents, which may vary markedly in their pharmacological actions. Some of these alkaloids are useful as therapeutic drugs, such as morphine, while many others are not (see Fig. 1 for details). The five major alkaloid constituents of opium can be separated into essentially two groups, based upon differences in their chemical structure. The narcotic analgesics morphine and codeine, as well as the convulsogenic compound thebaine, are phenanthrene derivatives. Although thebaine is not an analgesic, it is used as a chemical precursor in the development of many clinically useful semisynthetic opioid compounds such as oxycodone (Percocet®). The benzylisoquinolone derivatives of opium include the phosphodiesterase inhibitor and smooth muscle relaxant papaverine and the antitussive compound noscapine. However, of all the compounds contained in opium, the major alkaloid that is almost exclusively derived from the plant is morphine.

Originally, the term ‘opioid’ was coined by Acheson (Martin, 1967) to designate drugs whose actions resemble morphine, but that may be chemically distinct from its phenanthrene structure. This definition has now been broadened to include morphine receptor antagonists, as well as agonists, that have a wide spectrum of action on the opioid system (see review by Martin, 1967).

In 1954, Beckett and Casy hypothesized that synthetic analgesic opioid drugs and related morphinans ‘fit’ at a receptor surface stereospecifically. It was through this interaction at the receptor surface that analgesia resulted. It was not until the 1970s, with the advent of biochemical radioligand-binding studies, that the agonist-opioid receptor relationship could be probed. These studies were now feasible since they could be conducted with either radiolabelled opioid receptor antagonists such as naloxone or radiolabelled opioid narcotic agonists such as levorphanol. By measuring stereospecific binding in the presence of nonspecific interactions, the identity of opioid receptors in the brain of mammals was begun (Goldstein et al., 1971; Pert & Snyder, 1973).

In rats, it could be shown that electrical stimulation of various brain regions produced analgesia in animals and that naloxone could reverse the effect (Akil et al., 1974). Sub-

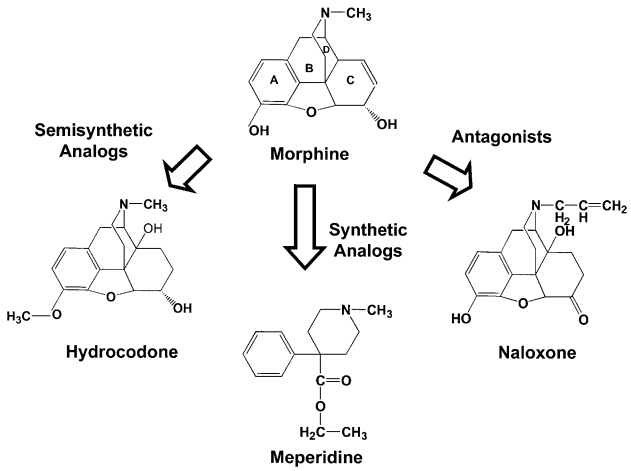

Fig. 1. Chemical structure of morphine, the major alkaloid constituent of the opium poppy *P. somniferum*, and some important chemically related semisynthetic and synthetic analogues. The basic chemical structure of morphine has also allowed for the synthesis of opioid receptor antagonists such as naloxone. The rings of the morphine structure are labeled, by convention, A (aromatic), B (cyclohexane), C (cyclohexene), and D (piperidine) to depict those regions of the molecule that constitute the phenanthrene nucleus (rings A, B, and C).

sequent to this, the development of bioassays and binding studies provided the necessary methods by which to screen extracts from both the brain and pituitary gland for opioid binding affinity. Hughes et al. (1975) showed that endogenous opioid peptides (EOPs) could be isolated from brain extracts and that these peptides possessed morphine-like properties. These EOPs include enkephalins, dynorphins, endorphins, and a large number of chemically distinct peptides derived from mammalian tissue (for reviews, see North, 1986; Pasternak, 1993; Fowler & Fraser, 1994). The effects of these opioid peptides are mediated via specific binding sites or receptors. Countless numbers of chemically synthesized non-peptide analogues mimic the actions of these peptides. These synthetic and semisynthetic opioid analogs are distinguished chemically as belonging to the morphinans, benzomorphans, phenylpiperidines, diphenylheptanes, and oripavines (North, 1986).

2. The classification of opioid receptors

The postulation of opioid receptor existence was stated in the pioneering work of Beckett and Casy (1954), which, in 1965, allowed Portoghese to theorize the existence of separate opioid receptors by correlating analgesic activity to the chemical structure of many opioid compounds.

Martin et al. (1976), using congeners of morphine, obtained the first in vivo evidence of opioid receptors. These workers identified three distinct syndromes produced by these agents in the chronic spinal dog. Thus, it was postulated that these syndromes were due to agonist interaction with three stereochemically related receptors. The morphine syndrome was mediated by the μ receptor, the ketocyclazocine syndrome by the κ receptor, and the SKF 10,047 syndrome by the σ receptor. For a complete profile of side effects for each syndrome see Martin et al. (1976). It should be noted that the σ receptor is no longer considered to be opioid in nature, and can no longer be classified as such since its physiological and pharmacological actions are not blocked by naloxone (Holtzman, 1980).

The development of in vitro pharmacological bioassays greatly enhanced the elucidation of heterogeneity between and within classes of opioid receptors. The mouse vas deferens and guinea pig ileum preparations were used by Hutchinson et al. (1975) to show that with ketocyclazocine and other related compounds, a consistently lower potency ratio for agonism was found in the mouse vas deferens when these same agents were compared in the guinea pig ileum. It was later shown by Lord et al. (1977) that there was a higher κ:μ ratio in the guinea pig ileum when compared with the mouse vas deferens. Thus, these tissues appear to have a heterogeneous population of opioid receptors. In addition to having μ and κ receptor populations, the mouse vas deferens subsequently was shown, using endorphins and enkephalins as agonists, to possess another profile of action for these agents that was independent on the other opioid receptors, giving rise to the postulation of the third opioid receptor called the δ receptor (Lord et al., 1977; Hutchinson et al., 1975).

Recently, many new opioid-binding sites have been postulated, including the β-endorphin selective ε receptor (Chang et al., 1984; Nock et al., 1990; for a review, see Pasternak, 1993). However, its characteristics have not been clearly defined, due to a lack of selective biochemical procedures and specific binding agents with which to label the site. However, with research comes change, and such change has included the development of molecular pharmacological techniques. These techniques no longer restrict research in this area to the use of selective opioid agonist and antagonist compounds to probe tissues rich in a particular opioid receptor subtype to delineate function. Instead, the search for novel opioid receptors, classification of receptor subtypes, delineation of the receptor pharmacology, or determination of receptor location in the CNS or peripheral organs such as the heart can be studied with isolated recombinant complementary DNA (cDNA). The isolated cDNA that genetically encodes the opioid receptor subtype to be investigated can be expressed either in opioid receptor naïve cell lines or in oocytes isolated from *Xenopus laevis* frogs. Recently, molecular cloning techniques have been used to identify the cDNA encoding an opioid-like G-protein-coupled receptor called ORL-1 (Meunier et al., 1995). Although this receptor shares marked structural similarity to the μ-, κ-, and δ-opioid receptors, it is unique such that it has a very low binding affinity for all synthetic and peptide opioid ligands (Reinscheid et al., 1995). Rather, it selectively binds an endogenous peptide that exhibits homology to dynorphin A, and has been called orphanin FQ or nociceptin (Meunier et al., 1995; Reinscheid et al., 1995; Nothacker et al., 1996).

Thus, as with many other receptor systems in pharmacology, many opioid receptors that had only been postulated in the past may now be characterized at the molecular and genetic level.

3. The molecular biology of opioid receptors

The initial cloning of opioid receptors by tissue protein isolation and purification methods did not prove to be successful. In order for such methods to be successful, proteins such as those that constitute the opioid receptor subtypes must be expressed at high levels in the tissue from which the protein is isolated. Since opioid receptors are not highly expressed in most tissues for molecular study techniques, including polymerase chain reaction (PCR) amplification, radioligand-binding assays, and DNA hybridization, screens were developed and utilized to obtain cDNA clones of the opioid receptors.

An understanding of the molecular structure of opioid receptors has resulted from elucidation of their DNA coding sequences, whose transcription results in translation of the receptor protein (Reisine & Bell, 1993; Raynor et al., 1994).

Although the tissue distribution of opioid receptor mRNA levels has not been completely characterized for all the subtypes, each subtype may be encoded by the same multigene family. This is suggested, for example, since many separate cDNA clones have been reported for the δ-opioid receptor (Evans et al., 1992). Whether or not these are tissue-specific remains to be determined. However, regardless of whether cDNA isoforms exist, each opioid receptor RNA encodes a protein of which the primary sequence has been deduced. From the primary sequence, structural molecular models of the opioid receptors have been developed (see Fig. 2). The μ-opioid receptor is structurally similar to both the cloned κ (Yasuda et al., 1993) and δ (Evans et al., 1992) receptors, and all are members of the highly homologous superfamily of G-protein-coupled, seven transmembrane domain-spanning receptors (Reisine & Bell, 1993). Alignment of the primary amino acid sequences of the three receptors suggests that a high degree of homology exists between certain regions of the receptors. An examination of transmembrane-spanning regions II, III, and VII suggests that these are highly conserved, while regions I, IV, and V are less conserved (Knapp et al., 1995). A comparison of the primary sequence encoding the intracellular regions of the receptors suggests that both the second and third loops are highly conserved amongst opioid receptor subtypes. These areas are proposed to mediate G-protein-coupled intracellular actions (Dohlman et al., 1991). Application of the known sequence information for these proteins to molecular modeling programs has allowed for a deduction of computational models of the receptor structure (Pogozheva et al., 1998). Several features common to opioid receptors are required for agonist and antagonist binding. In addition to hydrogen binding and Van der Waals interactions between the receptor protein and opioid compounds is the reported presence of a structural binding cavity. This central binding cavity is thought to accommodate the piperidine ring moiety of the opioid molecule (ring D of the structure of morphine in Fig. 1). The presence of a flat surface also maximizes contact area between the protein and the aromatic portion of the opioid molecule. The last feature is the high degree of negatively charged amino acid residues within the extracellular domains of the protein that allow for a charge–charge interaction between the receptor protein and cationic sites present on the opioid molecule (Knapp et al., 1995; Meng et al., 1995).

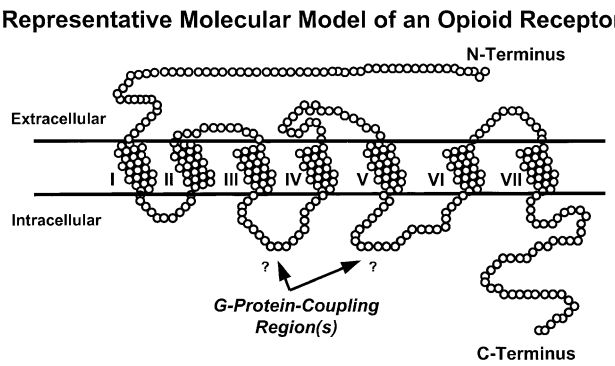

Fig. 2. Schematic diagram of the proposed molecular structure of opioid receptors present in the CNS. This model depicts the transmembrane folding of the opioid receptor protein based on hydropathy plot analysis of the deduced primary amino acid sequences of the μ-, κ-, and δ-opioid receptor clones. The structural motif of the receptor consists of seven helical transmembrane-spanning regions separated by extracellular and intracellular loops. These structural, as well as functional, properties of the opioid receptor are characteristic of the family of G-protein-coupled receptors. The domains of the receptor are indicated as I–VII. The experimentally determined intracellular sites of G-protein binding are suggested (see Reisine & Bell, 1993; Kong et al., 1994; Knapp et al., 1995).

3.1. The μ-opioid receptor

The μ-opioid receptor was cloned using a cDNA screening method. This method utilized a PCR-generated fragment of the rat δ-opioid receptor to probe a rat brain cDNA library (Chen et al., 1993). This method resulted in the cloning of a rat μ-opioid receptor 398 amino acids in length that shares 58% sequence homology with the δ-opioid receptor and 67% sequence homology with the κ-opioid receptor (Reisine & Bell, 1993). Expression of the cloned μ receptor in various cell systems reveals that it retains the pharmacological characteristics of the endogenous μ-opioid receptor in terms of agonist- and antagonist-binding affinities and coupling to adenylate cyclase (Chen et al., 1993). Tissue hybridization studies suggest a localization of μ-receptor mRNA in the basal ganglia and the thalamus, areas of the brain associated with neuronal pain pathways (Wang, J. B. et al., 1993). Additionally, these receptors are found to be widely expressed in peripheral tissues, such as the lungs, small intestine, large intestine, adrenal glands, kidneys, and spleen, using template-repeated PCR methods (Wittert et al., 1996). Localization studies for μ-opioid receptors in cardiac tissue largely suggest that this opioid receptor subtype is not expressed in this tissue (Krumins et al., 1985; Wittert et al., 1996). Thus, perhaps for this reason, there has been little research examining the role of opioid receptors in heart or cardiovascular function. Perhaps though, a more comprehensive study is warranted to delineate whether the μ-opioid receptor is truly totally absent from the heart. Although it may not be found in abundance on the surface of atrial or ventricular myocytes, it may be present in cardiac nerves or on endothelial cells of the cardiac vasculature. Delineation of the presence of this receptor on the coronary vasculature may provide information of therapeutic importance, especially for opioid overdose and resulting cardiac-associated toxicities.

Despite an understanding of the molecular nature of the individual opioid receptors, the advanced molecular studies to date have not provided for a concrete delineation of subtypes of the μ receptor, μ_1 and μ_2, originally suggested to mediate supraspinal and spinal analgesia (Pasternak & Wood, 1986), respectively. Recently, homologous recombination in mice has resulted in the disruption of the gene

that encodes the μ-opioid receptor. These mice lack the μ receptor, i.e., they are gene knock-out mice (Sora et al., 1997; Matthes et al., 1996; for a review, see Kieffer, 1999). The loss of the μ-opioid receptor results in a loss of the pharmacological actions of morphine and related μ agonists and antagonists alone, thus providing a novel model with which to explore not only the neuronal, but also the cardiovascular, actions of opioid compounds, and their relationship(s) centrally and peripherally. To date, no studies have been conducted to investigate such a relationship.

3.2. The κ-opioid receptor

The cloning of the κ-opioid receptor derives from studies initially directed towards screening of cDNA probes for somatostatin receptor subtypes (Meng et al., 1993). The sequence similarity to the mouse δ receptor, subsequent transfection of the cDNA clone into opioid receptor naïve cells, and expression of receptors with high-affinity binding for the selective κ agonist [^{3}H](5α,7α,8β)-(+)-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]-dec-8-yl]-4-benzene-acetamide methane sulphonate (U-69,593) confirmed the existence of this opioid receptor in the guinea pig (Xie et al., 1994). The guinea pig κ receptor (which shares 90% homology with the mouse and rat κ receptors) is a protein of 380 amino acids with 61% sequence homology to the δ receptor. Structurally the κ receptor shows the greatest similarity in sequence to the μ receptor in transmembrane-spanning regions II, III, and VII, as well as the second intracellular loop. The pharmacological actions of the cloned κ receptor have been shown to be similar to those of the endogenously expressed κ_1 receptor in the CNS (Yasuda et al., 1993). An examination of the tissue distribution of the mRNA for the κ receptor in several species shows that it is expressed in great abundance in the mouse brain and has also been detected in the heart. In the guinea pig, the distribution of κ receptor mRNA at four locations in the brain closely parallels receptor distribution as determined by radiographic techniques (Xie et al., 1994). The cloning of κ receptors from the mouse, rat, and guinea pig (Yasuda et al., 1993; Minami et al., 1993; Nishi et al., 1993; Xie et al., 1994; Xue et al., 1994) has allowed for their use as probes to screen human genomic libraries. The results of such genomic screens for these cloned κ receptors now includes human embryonic kidney cells (Lai et al., 1995) and the human placenta (Mansson et al., 1994). However, while we now know that κ receptors can be conclusively determined in tissues, there is still a necessity to clearly characterize the effects these receptors mediate in these tissues.

Recently, Simonin et al. (1998) disrupted the κ-opioid receptor gene in mice by gene targeting, and showed that antinociceptive actions of the selective κ receptor agonist trans-(±)-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methane sulphonate (U-50, 488H) in vivo were dependent upon the product of this gene. These results suggest that the κ_1 receptor subtype is the expressed gene product of the wild-type κ-opioid receptor gene. The genetic origins of the remaining putative κ receptor subtypes have yet to be identified.

3.3. The δ-opioid receptor

The mouse δ-opioid receptor was actually the first of the opioid receptors to be cloned (Kieffer et al., 1992; Evans et al., 1992). It is a 372 amino acid protein, whose sequence closely resembles that of related opioid receptors (Reisine & Bell, 1993). As with all the cloned opioid receptors to date, the molecular properties of the cloned δ receptor are similar to those found expressed in the CNS (Knapp et al., 1994). Studies have examined the tissue distribution of the mRNA for the δ receptor, and suggest that it is found in the lungs, adrenal glands, stomach, small and large intestine, kidneys, spleen, and sex organs (Wittert et al., 1996). However, compared with these organs, it was most abundant in the heart (Wittert et al., 1996; Zhu et al., 1998). The δ receptor, defined by Kosterlitz as the enkephalin-preferring receptor (Lord et al., 1977), may have two subtypes that are distinguished by the agonists [D-Pen^2, D-Pen^5]-enkephalin and [D-Ala^2]-deltorphin II as δ_1 and δ_2, respectively (Mattia et al., 1991). The pharmacological action of these sites may be related to both spinal and supraspinal analgesia, respectively (see Pasternak, 1993). Xu et al. (1993) has proposed two additional subtypes of the δ receptor, δ_{CX} and δ_{NCX}. In their proposed subdivision, the δ_{CX} receptor is thought to associate (or heterodimerize) with either the μ or κ receptor, forming an opioid receptor complex, while δ_{NCX} forms no associated complexes. Recently, biochemical and pharmacological evidence has revealed that heterodimerization occurs between opioid receptors, such as between μ and δ (George et al., 2000) and κ and δ (Jordan & Devi, 1999). While the κ–δ heterodimer exhibits both functional and ligand-binding properties that are distinct from either of its monomeric receptors, the co-expression of μ- and δ-opioid receptors has been shown to change the affinity state for the μ receptor (but not the δ receptor), suggesting a possible mechanism for the synergistic effects of opioid agonist drugs for these receptors in analgesia (Martin & Prather, 2001), which may be extrapolated to include effects observed in the heart. The heterogeneity of opioid receptors, therefore, exists not only at the molecular level within and between tissue, but is also reflected in the diversity of location and disparity between species (Martin, 1984; Mansour et al., 1988). Therefore, while distribution remains consistent with the role of the receptor in physiological function, synergistic activity between receptors may be of greater importance than originally proposed.

Thus, despite the introduction of the cloned opioid receptors, their present classification remains based primarily upon the pharmacological profiles of numerous opioid agonists as they relate to different potency ratios in bioassays, differences in drug-binding profiles, differential effects of naloxone and other selective antagonists, and simply how the receptor relates to various physiological actions, be it in

Table 1
A list of opioid receptor agonist and antagonist drugs for the μ-, κ-, and δ-opioid receptors

Opioid receptor	Agonist	Antagonist
μ	Morphine	Naloxone
	Fentanyl	Naltrexone
	Levorphanol	β-Funaltrexamine[1]
	Methadone	Nalbuphine[2]
κ	U-50,488H	Nor-BNI
	EKC	MR-2266
	U-62,066E (spiradoline)	Naloxone
	U-69,593	
δ	(−)TAN-67	Naltrindole
	SIOM[3]	BNTX
	SNC 80[4]	Naltriben

[1] β-FNA is an irreversible opioid receptor antagonist.
[2] Nalbuphine is an opioid receptor antagonist with partial agonist properties.
[3] 7-Spiroindinooxymorphone.
[4] (+)-4-[((αR)-α(2S,5R)-4-allyl-2,-5-dimethyl-1-piperazinyl)-3-methoxy-benzyl]-*N*,*N*-diethylbenzamide chloride.

the CNS or in peripheral organs (see Table 1). While these studies have effectively supplemented previous experimental evidence of receptor function, they may also provide a relationship between opioid receptors and human disease. An understanding of the role of opioid receptors under normal and pathological states, especially of the heart, may promote more vigorous investigation that accurately determines the location and function of these receptors and the action of agonist and antagonist drugs. As of yet, only few investigations addressing the role of opioid receptors in heart disease are being conducted.

4. Endogenous opioid peptides in the heart

Myocardial cells are capable of the synthesis, storage, and release of peptides, such as atrial natriuretic peptide (De Bold, 1985) and opioid receptor peptides (Barron et al., 1995). Opioid receptor peptides may be either secreted from nerves that innervate the heart or produced in myocardial tissue. Regardless of the manner of production, these peptides are devoid of activity until enzymatic proteolysis of the precursor by convertases results in one (or more) active peptide products.

Four major classes of EOPs exist and have been shown to be distributed throughout cardiac nerves, intrinsic nerves of the gastrointestinal tract, and in cardiac muscle. EOPs derive from four prohormone precursors: pro-enkephalin, pro-opiomelanocortin (POMC), pro-dynorphin, and pro-nociceptin. Table 2 describes some of the peptide products derived from these prohormones.

While our understanding of peptide processing in neurons is relatively complete, our understanding of the biochemistry of peptide processing in peripheral tissue such as cardiac muscle is not. It has been established that cardiac myocytes tend to contain a greater ratio of larger molecular weight peptides (such as the extended form of met-enkephalin, Met[5]-enkephalin-Arg[6]-Phe[7] or MERF, and dynorphin A), compared with neurons (Barron, 1999).

As with EOP distribution in the CNS, cardiac opioid peptide distribution is also highly variable within cardiac tissue. The processing of pro-enkephalin differs in the atria compared with the ventricles, and results in higher levels of MERF in ventricular tissue (Barron et al., 1995). Millington et al. (1999) recently characterized POMC peptide immunoreactivity in rat hearts using reverse-transcript PCR methods and found that in adult rat hearts, processing of POMC mRNA to the cardiac β-endorphin peptide (a truncated transcript that has no opioid receptor activity) is predominantly found in atrial tissue and not in either the ventricle or in cardiac nerves. In neonatal rat hearts, full-length POMC mRNA was found predominantly unprocessed. These studies suggest that cardiac muscle is biochemically competent in the synthesis of opioid peptides; however, a definite function for the peptides remains to be elucidated.

In neurons, opioid peptides are found in vesicles. Endorphins are discretely localized in internal neuronal structures, while enkephalins are widely distributed throughout the neuron. In cardiac tissue, enkephalins are present in vesicles within the atria, whereas they are found 'unpackaged' in the cytosol in ventricles (Barron et al., 1995; Barron, 1999; Millington et al., 1999). Unlike the POMC precursors of the endorphins, the biosynthesis of pro-enkephalin and pro-dynorphin occurs predominantly in ventricular cardiac myocytes, not in the atria. Dynorphin- and α-neoendorphin-immunoreactive postganglionic sympathetic nerve fibers innervate coronary blood vessels and cardiac muscle (Wegener & Kummer, 1994). Thus, EOPs are widely distributed in the heart.

Table 2
The EOP precursors and some of their active opioid peptide products involved in cardiovascular regulation and function

Precursor	Opioid peptide	Receptor[1]
Pro-enkephalin[2]	[Met]-enkephalin	$\delta > \mu >>> \kappa$
	[Leu]-enkephalin	
POMC[3]	β-Endorphin	$\mu \approx \delta >> \kappa$
Pro-dynorphin[4]	Dynorphin A	$\kappa >>> \mu > \delta$
	Dynorphin $A_{(1-8)}$	$\kappa >> \mu > \delta$
	Dynorphin B	$\kappa >>> \mu > \delta$
Pro-nociceptin	Nociceptin	ORL_1

[1] The efficacy of the opioid peptide to its receptor is only qualitatively described in this table for the μ-, κ-, and δ-opioid receptor subtypes found in the CNS (Fowler & Fraser, 1994).
[2] An additional opioid peptide product from this precursor that exhibits relevant cardiovascular actions is MERF (Zhang et al., 1996).
[3] An additional POMC peptide cleavage product is adrenocorticotropic hormone, which is converted to α-melanocyte-stimulating hormone-related peptides in cardiac tissue (Millington et al., 1999).
[4] Additional active peptides include leumorphin, α-neoendorphin, and β-neoendorphin (Fowler & Fraser, 1994).

The EOP system consists of the peptides endorphin, dynorphin, and enkephalin and their associated μ-, δ-, and κ-opioid receptors. These opioid peptides and receptors are widely distributed in the body, and all have complex actions in the heart. In the heart, κ and δ receptor agonists have been shown to inhibit atrial, but not ventricular, contractility, while μ agonists have been shown to inhibit ventricular contractility, without altering atrial function (Mantelli et al., 1987; Barron, 1999). A study of the actions of peptides on both electrical and mechanical properties of the isolated rat heart has shown that δ- and κ-opioid receptor agonists can directly depress cardiac function (Vargish & Beamer, 1989). The rate of vagal firing has also been shown to be regulated by opioid peptides (Pokrovsky & Osadchiy, 1995). Vagal bradycardia is inhibited by the administration of the intrinsic cardiac opioid heptapeptide MERF, presumably by the activation of δ-opioid receptors on prejunctional cardiac vagal nerves or parasympathetic ganglia, reducing acetylcholine release (Caffrey, 1999). Therefore, EOPs can mediate direct and indirect actions in various regions of the heart. Thus, in addition to complex differences in the general tissue distribution of opioid receptors, cardiac opioid peptide function is complicated by the fact that receptor expression is modulated by both changes in physiological states (such as hypotension) and disease (such as hypertension or heart failure) (Krumins et al., 1985; Dumont & Lemaire, 1988; Barron, 1999). The differential tissue distribution of opioid peptides, their receptors, and their function suggests that they may play a role in local neuronal and cardiac tissue homeostasis.

5. The CNS actions of opioids relate to actions in the heart

Morphine is a potent analgesic that is used in the treatment of serious pain, such as that associated with congestive heart failure (CHF) or heart attacks. When opioid drugs are administered, they may produce a variety of responses within the body including changes in cardiovascular function that are dependent upon opioid receptors (Martin, 1984). However, some responses have been shown to be opioid receptor-independent (for a review, see Pugsley et al., 1993a).

Intracellular recordings made from guinea pig substantia gelatinosa neurons in brain tissue have shown that κ-opioid agonists at low concentrations (10–100 nM) *augment* the voltage-dependent K^+ current (Grudt & Williams, 1993). While this effect is antagonized by naloxone, it provides not only a potential mechanism by which these compounds mediate antinociceptive properties (Grudt & Williams, 1993; Moore et al., 1994), but also a link between the opioid receptor and an ion channel. In hippocampal neurons, low doses of κ-opioid agonists (20–100 nM) also augment the neuronal voltage-dependent K^+ current known as the M current, while at high concentrations (1000–1500 nM), these κ-opioid receptor agonist drugs *block* the current (Moore et al., 1994). Further molecular biological studies show that *Xenopus* oocytes express κ-opioid receptor-specific binding sites when injected with the mRNA encoding the opioid receptor (Henry et al., 1995). When these same cells are additionally injected with mRNA that codes for a G-protein-linked, inwardly rectifying K^+ channel, activation of the κ-binding site by low concentrations of U-69,593 results in the development of a large K^+ current. This increase in current is blocked by the κ-opioid receptor antagonist norbinaltorphimine (nor-BNI). Thus, the evidence presented in these studies implies that the opioid receptor may be linked to a channel by an unspecified mechanism.

Studies with opioid agonists and antagonists in neuronal tissue suggest that in addition to an opioid receptor-mediated action, there is also a non-opioid receptor-mediated action of these compounds on ion channel function (for reviews, see North, 1986; Pugsley et al., 1993a). Voltage-gated Na^+ channels are responsible for the initiation of membrane depolarization and the conduction of action potentials in electrically excitable cells, resulting in contraction of the heart or transmission of electrical impulses in nerves. K^+ channels are responsible for repolarization of the cell membrane and cessation of action potentials in excitable cells. Morphine and naloxone have been shown to block the propagation of action potentials in many nerve and cardiac muscle preparations by directly inhibiting voltage-dependent Na^+ and K^+ currents (Frazier et al., 1973; Carratu & Mitolo-Chieppa, 1982). Alzheimer and ten Bruggencate (1990) demonstrated that the κ-opioid receptor agonists U-50,488H and U-69,593 had local anaesthetic actions in neuronal tissue that could not be reversed by opioid receptor antagonists. These opioid receptor-independent actions in neuronal tissue have also been observed with κ-opioid receptor compounds in cardiac tissue. Pugsley et al. (1994) examined the electrophysiological actions of the κ-opioid agonist U-50,488H, and showed that it blocked Na^+ and K^+ channels in isolated cardiac myocytes. Na^+-channel block resulted from an interaction of U-50,488H with the inactivated state of the cardiac Na^+ channel in the absence and presence of naloxone.

Thus, it is the result of such an extensive body of work with opioid receptors that has provided the foundation on which the remainder of this review will focus. While this review will attempt an overview on the cardiovascular actions of opioids, it will focus specifically upon several current areas of research that involve opioid receptor-dependent and -independent mechanisms. Specific attention will be given to the κ-opioid receptor agonist drugs, where applicable, since these compounds have been the focus of the author's research for the past decade.

6. The cardiovascular actions of opioids

At clinically relevant doses, the cardiovascular actions of morphine and related narcotic analgesics are limited. The

role that endogenous or exogenously administered opioids, especially κ agonists, play in the regulation of this system is difficult to determine because the highly influential physiological effects they impart depend upon pharmacological variables, such as dose, site, and route of administration; receptor specificity; and species. To further complicate matters, the actions opioids exhibit are mediated both by opioid receptors located centrally in specific areas of the brain and nuclei that regulate the control of cardiovascular function and peripherally by tissue-associated opioid receptors. In the periphery, the locus of opioid peptide-secreting neurons and opioid receptors has been defined more clearly for some organ systems (such as in the gastrointestinal tract) than others (such as the heart and smooth muscle of the vasculature). While no consensus has been established regarding the role that opioids play in the regulation of the cardiovascular system, they have been implicated in several significant cardiovascular disease states. The following sections will attempt to review the general role of opioids on the cardiovascular system, as well as their involvement in hypertension, CHF, ischaemic preconditioning, and ischaemic arrhythmogenesis.

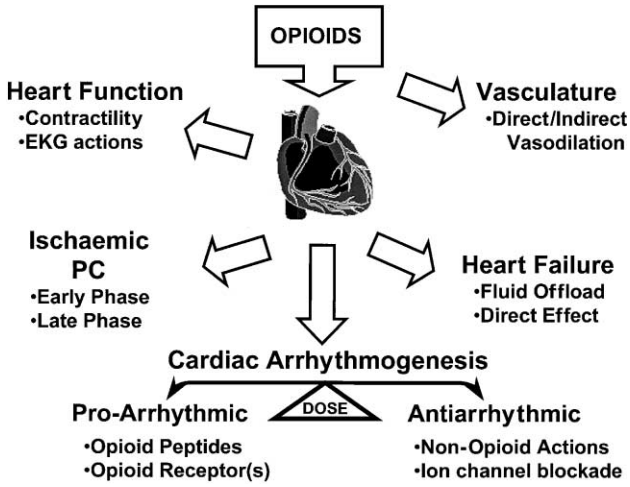

Fig. 3. Some of the actions of opioids on the heart and cardiovascular system. Opioid actions may either involve direct opioid receptor-mediated actions, such as the involvement of the δ-opioid receptor in ischaemic preconditioning or indirect, dose-dependent, non-opioid receptor-mediated actions such as ion channel blockade associated with the antiarrhythmic actions of opioids, especially arylacetamide drugs (see text for details).

6.1. The general cardiovascular actions of peptides and non-peptide opioids

The κ agonists, and all opioids in general, exhibit a variety of complex pharmacological actions (see Fig. 3) on the cardiovascular system (Holaday, 1983). The CNS effects of κ agonists such as analgesia are clearly mediated by opioid receptors (Von Voigtlander et al., 1982; Lahti et al., 1982; Leighton et al., 1987; Kunihara et al., 1989, 1993), but the actions of these compounds and, hence, role of the opioid receptors in peripheral tissues is less well established.

The presence of opioid receptors and their importance in cardiac tissue, as well as their involvement in cardiac function, is now less uncertain than it has been in the past. Hughes et al. (1977) were the first to show that endogenous enkephalins occur in rat and rabbit atria. Less uncertainty exists as to the localization of EOPs in the heart (see Section 4), and it is generally agreed that opioid receptors are differentially distributed between atria and ventricles (Holaday, 1983; Lang et al., 1983; Krumins et al., 1985; Weihe et al., 1985; Tai et al., 1991; Barron et al., 1992). The highest specific receptor density for binding of κ agonists, such as U-69,593 and diprenorphine, was in the right atrium (Krumins et al., 1985; Tai et al., 1991); binding was the least in the left ventricle. The development of a novel, selective κ_2 peptide ligand, MERF, has confirmed the presence of at least two κ receptor-binding sites, κ_1 and κ_2, in cardiac muscle (Zhang et al., 1996). In addition to κ receptor binding in the heart, studies also confirm the existence of the δ-opioid receptor (Krumins et al., 1985; Ventura et al., 1989) and, most recently, the presence of high-affinity nociceptin-binding sites (Dumont & Lemaire, 1998) in the rat heart. As with the κ-opioid receptor, the distribution of the δ-opioid receptor favors atrial tissue and the right side of the heart more than the left side of the heart (Barron, 1999). The data confirming the presence of the μ-opioid receptor in the heart are less conclusive (Krumins et al., 1985; Ventura et al., 1989; Tai et al., 1991), and studies conclude that this opioid receptor subtype is not present in cardiac tissue (Ela et al., 1997). Whether or not these differences in the presence of opioid receptors or their distribution within the heart are of importance cannot be judged, as it has not been shown whether these receptors are differentially located in cardiac muscle or cardiac nerves, or are expressed on immunomodulatory cells within the heart. What we can surmise from these studies is that opioid receptor binding studies in the heart are hampered both by the lack of appreciable ligand binding to cardiac membrane fractions and the presence of low numbers of receptors in the cardiac muscle. While these studies characterize the global presence of opioid receptors in cardiac muscle, their involvement and distribution within critical regions of the heart involved in conduction, such as the His-Purkinje and nodal tissue, are not known.

The physiological significance of peripheral opioid receptor distribution in the vasculature is also not fully understood. Peripheral binding of EOP, arylacetamides, and other opioid agonists should provide an outline of the involvement of the opioid system in haemodynamic function and cardiac activity, in addition to their actions on the cardiovascular control centres found in the brain (Lang et al., 1983).

The effects of κ agonists, such as U-50,488H and related arylacetamides (Szmuszkovicz & Von Voigtlander, 1982), on blood pressure have been examined (see Pugsley et al., 1992a, 1993a, 1993b). These actions are dose-, species-, and route-dependent. U-50,488H, for example, displays a markedly different cardiovascular profile when injected intravenously,

as compared with injection directly into the CNS (Feuerstein et al., 1985; Pugsley et al, 1992a, 1993b). In anaesthetized dogs, κ agonists, such as U-50,488H and (5α,7α,8β)-(±)-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]-dec-8-yl]-4-benzeneacetamide hydrochoride [U-62,066E (spiradoline)], produce dose-related decreases in blood pressure, heart rate, peak systolic pressure, and cardiac contractility when administered intravenously. The cardiovascular responses to both opioid agonists were abolished by the previous administration of naloxone (Hall et al., 1988). Early studies with morphine and related μ-opioid receptor agonists showed that these agents depress blood pressure, an action that could be blocked by antagonists, such as naloxone and levallorphan (Martin, 1967). It should be re-emphasized that the cardiovascular-depressant actions of these opioid receptor agonist drugs tend to occur at doses that would not be deemed 'clinically relevant,' but rather, are mediated by a mechanism that may be independent of opioid receptors. Thus, these opioid receptor-independent actions reveal novel avenues for the pharmacological exploration of opioid agonist and antagonist drugs.

In addition to causing a reduction in blood pressure, U-62,066E, U-50,488H, and related κ agonists all dose-dependently reduce the heart rate in anaesthetized rats (see Pugsley et al., 1993a). This is suggestive of an effect on either the reflex mechanisms that regulate the heart rate during hypotension or direct actions on the electrical or mechanical properties responsible for normal cardiac contractility. U-50,488H and U-62,066E cause slight depressant actions on the heart rate and blood pressure at low doses (Pugsley et al., 1992a, 1998). Neither the opioid antagonists (−)-5,9α-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan hydrochloride (MR2266) nor naloxone reduced the cardiovascular actions of the arylacetamides, suggestive of a lack of involvement of opioid receptors and revelation of a possible direct effect of these arylacetamide drugs on cardiac muscle.

In intact animals, opioid receptor-dependent effects in the CNS, as discussed in Section 5, may mediate the cardiovascular actions produced by opioid agonists. A recent example of opioid-mediated regulation of heart rate was shown by Uchida et al. (1999). This study showed that morphine, when administered intraspinally, depresses somatocardiac sympathetic reflexes. However, when administered supraspinally, these reflex responses were enhanced. This study suggests that morphine (or more specifically, the μ-opioid receptor)-mediated regulation of heart rate may occur within the CNS, and that some variability exists in its regulation.

In order to examine the pharmacological actions of opioid compounds that occur independent of opioid receptors, it is necessary to perform such studies, either in the presence of opioid antagonists such as naloxone or to use enantiomers, which lack opioid receptor agonist properties. In anesthetized rats, the cardiovascular responses to κ-opioid agonists, such as U-50,488H and U-62,066E, in the presence of opioid antagonists, such as naloxone or MR2266, were not changed (Brasch, 1986; Kaschube & Brasch, 1991; Pugsley et al., 1992a, 1995). It was concluded from these studies that since opioid receptor antagonists did not block these responses, they were not mediated by opioid receptors. In a study that examined the cardiovascular responses of a pair of κ-opioid receptor enantiomers, the inactive (R,R)-enantiomer, (+)PD129,289, of the selective κ-opioid receptor agonist (−)PD129,290 produced similar reductions in heart rate and blood pressure (Pugsley et al., 1993b), indicative of actions independent of opioid receptors.

As discussed briefly in Section 5, opioid receptor agonists and antagonists have been shown to block ion channels that constitute the genesis of the action potential in neurons. In addition to the opioid receptor-independent-mediated actions of U-50,488H and related κ agonists on blood pressure and heart rate (i.e., actions not blocked by opioid receptor antagonists), these drugs produce changes in the ECG indicative of a drug interaction with cardiac ion channels (Pugsley et al., 1992a, 1995, 1998).

The ECG changes produced by U-50,488H and related arylacetamides in the presence of effective opioid receptor blockade are suggestive of a drug interaction with cardiac ion channels (Pugsley et al., 1993a, 1995, 1998). Table 3 summarizes the actions of several arylacetamide κ-opioid agonists and their actions on blood pressure; heart rate; and the P−R, QRS, RSh, and Q−T intervals of the rat ECG. The ECG changes produced by these κ agonists at high doses are indicative of cardiac Na^+-channel blockade. In addition to P−R interval prolongation, the QRS width was also widened in rats. These drugs also produced an increase in amplitude of the RSh measure, an index of Na^+-channel blockade in the rat (Penz et al., 1992). However, despite the actions of these drugs on ECG intervals indicative of Na^+-channel blockade, there was also a widening of the Q−T interval, an

Table 3
D_{25} drug doses of arylacetamide κ-opioid agonists in intact rats: effects of on heart rate, blood pressure, and ECG measures

	ECG measures (msec)					
Drug	Heart rate	Blood pressure	P−R	QRS	RSh	Q−aT
U-50,488H	1.5	> 32	20	> 32	16	32
U-62,066E	4.0	8.0	15	25	2.0	10
PD117,302	5.5	0.50	3.0	7.5	1.0	6.0

The non-opioid actions of structurally related arylacetamide κ-opioid receptor agonists were examined in pentobarbital-anesthetized rats. Heart rate, blood pressure, and ECG measures were determined as D_{25}, the dose (μmol/kg/min, i.v.) producing a 25% change in the given response. This measure allowed for a determination of the differential actions of the arylacetamides on ECG measures, an index of drug action on cardiac ion channels (n = 6). The drug dose produced a 25% change from control in intact animals for 6 determinations per measure. All drugs consistently reduced the heart rate, but had varying D_{25} doses for blood pressure reduction. PD117,302 ((±)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl] benzo[b]-thiophene-4-acetamide monohydrochloride), produced evidence of Na^+-channel blockade (changes in the P−R, QRS, and RSh measures) and K^+-channel blockade (changes in the Q−aT interval) at lower D_{25} values than either of the other two drugs.

index of cardiac repolarization, suggestive of K^+-channel blockade. Other studies propose that Na^+-channel blockade can occur with opioid agonists and antagonists (Frame & Argentieri, 1985; Sarne et al., 1991), and it is the suggested mechanism by which opioids may mediate observed pharmacological actions in cardiac muscle.

Studies conducted in rat hearts complement both cellular and in vivo observations described above. The effects of U-50,488H and other κ agonists have been examined in the Langendorff isolated rat heart, and similar changes in ECG intervals were observed. These agents prolonged the P–R interval and QRS duration of the ECG, and reduced peak left-ventricular pressure in a concentration-dependent manner (Pugsley et al., 1992a). Pretreatment of hearts with naloxone at an effective opioid receptor blocking concentration (1 μM) did not prevent the actions of these opioid agonists (Pugsley et al., 1992a, 1992b, 1998). This non-opioid action on the ECG suggests that these compounds block Na^+ and K^+ channels in a manner similar to Class I antiarrhythmics such as quinidine. Few other studies have been reported that examine the effects of opioid agonists on isolated heart function and that relate the observed effects to an interaction with cardiac ion channels. The only other report of these effects is by Xia et al. (1994), which shows that at a concentration of 1 μM, U-50,488H reduced heart rate and contractility. The majority of studies involving the opioid receptor-independent actions of κ receptor agonists have been conducted in isolated cardiac tissue. These opioid receptor-independent actions usually occur with the application of micromolar concentrations of opioid drugs to isolated cardiac tissue preparations, whereas κ-opioid receptor agonism usually occurs at nanomolar concentrations of these same opioid drugs.

Studies using a variety of cardiac muscle preparations have shown that a large number of diverse opioid agonists, such as morphine and U-50,488H, and antagonists, including naloxone and Mr1452, exert similar opioid receptor-independent properties on cardiac muscle (see Pugsley et al., 1993a). Thus, a large, diverse group of chemicals exist that are able to activate or block opioid receptors with relative specificity at low concentrations, yet at high concentrations produce common opioid receptor-independent actions on the cardiac tissue indicative of ion channel blockade (Rashid & Waterfall, 1979; Pugsley et al., 1995). In isolated guinea pig and rat atrial and ventricular muscle, opioids suppress excitability in cardiac muscle by both increasing the threshold for stimulation and reducing action potential amplitude (Rashid & Waterfall, 1979). Correspondingly, the maximum rate of depolarization is reduced by opioids in a manner consistent with Na^+-channel blockade (Alarcón et al., 1993). The results of studies with opioid drugs, such as sufentanil (Pruett et al., 1991) and piritramide, pethidine, and morphine (Helgesen & Refsum, 1987), corroborate the suggestion of an interaction of κ-opioids with cardiac K^+ channels. However, the opioid receptor-independent nature of the ion channel blockade that was exhibited is not restricted to Na^+ or K^+, and may also include L-type Ca^{2+} channels found in cardiac muscle. K^+-channel blockade was suggested on the basis of increased action potential duration (APD), especially at the terminal phase of repolarization, observed with many opioids (Brasch, 1986; Helgesen & Refsum, 1987; Hicks et al., 1992). The use of Ca^{2+} fluorescent techniques for measurement of cardiac myocyte contractility suggested that the negative inotropic actions of κ-opioid agonists may also be due to inhibition of L-type Ca^{2+} currents (Kasper et al., 1992; Lakatta et al., 1992).

The ion channel blocking or opioid receptor-independent actions of arylacetamides, κ agonist drugs, and other opioid compounds have been examined on various ionic currents evoked from many voltage-clamped cell types. The cell types include cardiac myocytes and cardiac ion channels expressed in isolated frog oocytes. While the literature describing opioid receptor-independent properties of opioid drugs is meager, since the majority of papers investigate the coupling of opioid receptors to ion channels via second messenger systems, duBell and Lakatta (1991) and Utz et al. (1995) have examined the effect of U-50,488H on the slow inward Ca^{2+} current of cardiac myocytes. The intracellular application of U-50,488H had no effect on the Ca^{2+} current (Utz et al., 1995), whereas extracellular application of U-50,488H, in the presence of naloxone, inhibited the current in a manner indicative of an opioid receptor-independent mechanism. While investigating the possible cardiotoxic effects produced by opioids in human poisoning, Wu et al. (1997) also found that opioids, such as pethidine and dextropropoxyphene, exert negative inotropic actions in cardiac muscle byv blockade of Ca^{2+} currents in myocytes in the presence of naloxone. In isolated rat cardiac myocytes, the κ-opioid receptor agonists U-50,488H, (−)PD129,290, and U-62,066E have all been shown to produce a concentration-dependent inhibition of the transient outward and sustained plateau K^+ currents (Pugsley et al., 1993b, 1994, 1995, 1998). Other studies with U-50,488H show that it also inhibits delayed rectifier K^+ currents (Utz et al., 1995). The weak μ-opioid receptor agonist propoxyphene produces naloxone-insensitive cardiotoxicities that include K^+-channel block (Ulens et al., 1999). The actions of propoxyphene have been examined in *Xenopus* oocytes that express the human ether-a-go-go-related gene (HERG) channel (Ulens et al., 1999). HERG is an ion channel that encodes the rapidly activating component of the delayed rectifier K^+ current responsible for initiating ventricular repolarization of action potentials in cardiac myocytes. Propoxyphene blocked HERG currents by slowing down channel activation and accelerating channel deactivation kinetics (Ulens et al., 1999). Thus, the expression of ion channels in an experimental oocyte model can be used successfully to precisely probe the receptor-independent effects of opioid agonists, since opioid receptors are not present on oocytes.

In addition to many different opioid agonist drugs blocking either Ca^{2+} or K^+ ion channels, these same drugs may block cardiac Na^+ currents. Na^+-channel blockade by mor-

phine occurs not only in rat myocytes, but also in human atrial myocytes (Hung et al., 1998). The electrophysiological characteristics of voltage-dependent Na^+-channel blockade in myocytes by κ-opioid agonist drugs, such as U-50,488H and U-62,066E, includes a hyperpolarizing shift in the voltage-dependence of Na^+-channel inactivation, a slowing in the rate of Na^+ channel recovery from inactivation, and marked frequency-dependent properties, which all indicate that these compounds exhibit a high affinity for the inactive state of the cardiac Na^+ channel (Pugsley et al., 1993b, 1998; Hung et al., 1998).

The heterogeneous expression of ion channels in oocytes is a clever method by which to examine the receptor-independent actions of κ-opioid receptor agonists such as U-50,488H (Pugsley et al., 2001). Fig. 4 depicts the actions of U-50,488H on rat cardiac Na^+ currents expressed in *Xenopus* oocytes. This figure reveals an effective blockade of Na^+ currents independent of any action on the κ-opioid receptor. These results confirm the ion channel blocking actions observed in vivo (Pugsley et al., 1993b, 1998) and in vitro (Pugsley et al., 1992a, 1994), and provide direct evidence that this opioid receptor agonist drug blocks Na^+ channels that are expressed in cardiac tissue. Thus, the mode of channel block produced by κ-opioid agonists in myocytes and oocytes is similar to the local anesthetic actions observed by Alzheimer and ten Bruggencate (1990) in neuronal tissue, which were not blocked by opioid receptor antagonists. These studies present the clearest evidence for the opioid receptor-independent actions of the κ-opioid receptor.

It should be noted briefly that in addition to an examination of the opioid receptor-independent cardiac effects of opioid agonist drugs, many studies have found opioid receptor-independent cardiac effects of opioid antagonists. The most extensively studied opioid receptor antagonist is naloxone, a benzomorphan opioid receptor antagonist at all opioid receptors (Martin, 1967). Naloxone has been shown to reduce the maximum rise rate of the cardiac action potential (Phase O) and to increase cardiac APD indicative of Na^+- and K^+-channel blockade, respectively, in both canine cardiac Purkinje fibers (Frame & Argentieri, 1985) and guinea pig papillary muscles (Brasch, 1986). Recently Pugsley and Goldin (1997) examined the opioid receptor-independent effects of racemic (±)-naloxone on rat cardiac and neuronal Na^+ currents expressed in oocytes isolated from *X. laevis* frogs. These studies showed that (±)-naloxone produced a direct, concentration-dependent block of both cardiac and neuronal isoforms of the Na^+ current in the absence of any actions the drug may have on opioid receptors.

Some of the receptor-independent actions of κ agonists that have been observed in cardiac tissue have also been observed in vascular tissue. The actions of a series of κ agonists were examined on the basilar and middle cerebral arteries of dogs by Altura et al. (1984). MR 2034 and U-50,488H dose-dependently contracted both cerebral arteries, and these contractions were not inhibited by naloxone. Illes et al. (1987) examined the actions of U-50,488H and ethylketocyclazocine (EKC) on stimulation-induced contractions of isolated rat tail veins. Concentration-dependent depression of contractility by these opioids was not prevented by naloxone. In addition to κ agonist drugs, other opioids such as fentanyl have also been found to produce biphasic changes in basal canine epicardial coronary artery ring tension that is not blocked by naloxone, but is blocked by Ca^{2+}-channel blocking drugs such as nifedipine, suggesting a concentration-dependent opioid receptor-independent modulation of intracellular Ca^{2+} (Introna et al., 1995). The

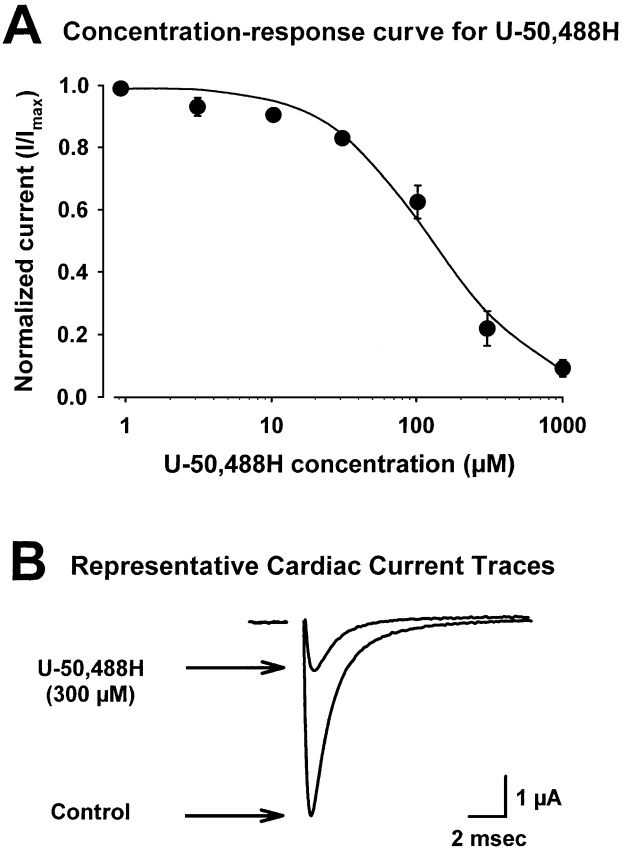

Fig. 4. **A**: Concentration–response relationship for the opioid receptor-independent action of the κ-opioid receptor agonist U-50,488H on Na^+ currents expressed in ***X. laevis*** oocytes. Currents were recorded by two-electrode whole-cell voltage clamp. Oocytes were injected with 50 ng of in vitro transcribed RNA encoding the cardiac Na^+ channel α-subunit. After 2 days of incubation at 20°C in ND-96 solution, currents were evoked by depolarizing the cell to −10 mV from a holding potential of −120 mV at 6-sec intervals. The data were filtered at 3 kHz. U-50,488H (1–1000 μM) was applied in the bath for 5 min at a flow rate of 1–2 mL/min, and the peak Na^+ current measured. The data are shown as peak current normalized to the maximum current in the absence of U-50,488H. The line describes the best fit for the Hill equation $I_{Na} = 1/(1 + [A]/EC_{50})^n$, where I_{Na} is the fraction of current remaining after block by U-50,488H and n is the Hill coefficient that describes the stoichiometry of the drug-channel interaction. For U-50,488H block of the cardiac Na^+ current, the EC_{50} was 130 ± 39 μM and the Hill coefficient 1.2 ± 0.3 (mean ± SD for 5 oocytes). **B**: Representative Na^+ current traces evoked in oocytes expressing the cardiac Na^+ channel, as described for A. The oocytes were depolarized to −10 mV from a holding potential of −120 mV in the absence (control) and presence of 300 μM U-50,488H.

effects of morphine, U-50,488H, EKC, and bremazocine have been examined on coronary arteries (el Sharkawy et al., 1991). These authors also found that coronary artery relaxation by these opioid drugs could not be blocked by either naloxone or MR2266, but could be blocked by verapamil. Harasawa et al. (1991) examined the actions of U-62,066E (spiradoline) on porcine coronary arteries, and found that naloxone did not inhibit the re-admission of Ca^{2+} back into arteries exposed to a Ca^{2+}-free medium. The re-admission of Ca^{2+} back into coronary arteries, measured by the intracellular fluorescent Ca^{2+} indicator fura-2 was completely prevented by U-62,066E. Thus, these studies in isolated vascular tissue suggest that opioids mediate an opioid receptor-independent relaxation of tissue that can be attributed to inhibition of voltage-dependent Ca^{2+} entry into smooth muscle cells. Thus, while the importance of high-dose, opioid receptor-independent agonist behaviour in isolated tissues may be limited, these findings concur with those in isolated myocytes and heterogeneous ion channel expression in oocytes, that opioid receptor agonists may directly interact with voltage-dependent ion channels.

Thus, the Ca^{2+}-, K^{+}-, and Na^{+}-channel blocking actions of U-50,488H and other arylacetamide opioid agonists have been demonstrated in both neuronal and cardiac tissue. While the direct application of this information to a clinical setting currently may be limited only to the development of an understanding of the mechanism of toxicity associated with opioid overdose, these findings may, at a basic research level, provide additional structural information regarding the interaction of drugs with cardiac ion channels.

6.2. The role of peptide and non-peptide opioids in hypertension

High blood pressure is a condition associated with many serious complications, including cerebrovascular incidents and CHF. In most cases, the cause of the elevation in blood pressure is not known.

Many neuroanatomical regions within the brain regulate the cardiovascular system. Until recently, the brain areas that were generally considered to be regulatory included the ventral lateral medulla, nucleus tractus solitarius, lateral hypothalamus, and paraventricular nucleus. However, it is now suggested that the dorsal hippocampus, an integral constituent of the limbic (or emotional) system, may also be involved (Wright et al., 1999). Wang and Ingenito (1994) showed that the intrahippocampal injection of dynorphin $A_{(1-8)}$ produced a dose-dependent reduction in blood pressure and heart rate, and that these actions were mediated by the κ-opioid receptor. Similarly, the use of opioid receptor antagonists have shown that blockade of opioid receptors reduces the autoregulatory capacity of the hypothalamus, suggesting that EOP may be involved in the regulation of blood pressure (Komjati et al., 1996).

However, some studies suggest that opioids are not involved in the central regulation of blood pressure. The injection of μ-, δ-, and κ-opioid receptor agonists into the paraventricular nucleus, dorsal hippocampus, and rostral vertrolateral medulla of normotensive and spontaneously hypertensive rats suggested that only agonists for the μ- and δ-opioid receptors reduced blood pressure and heart rate (Sun et al., 1996). All cardiovascular responses produced by these opioid agonists were blocked by naloxone. Naloxone itself in these studies produced an increase in blood pressure and heart rate. Thus, these authors conclude that the obvious lack of selective activity of these agonists and antagonists in the normotensive and hypertensive conditions argues against a role for the involvement of EOP in hypertension (Sun et al., 1996). However, while the involvement of EOP may be in doubt, evidence has accumulated to suggest that the κ-opioid receptor may perform a significant role in blood pressure regulation.

McConnaughey et al. (1998) examined whether the effects of the chronic administration of the selective non-peptide κ-opioid receptor agonists U-50,488H and U-69,593 might alter opioid receptor levels or receptor affinity in those areas of the brain that regulate blood pressure. While these κ-opioid receptor agonists reduced blood pressure, no changes in κ-opioid receptor subtype distribution in the brain were observed with chronic administration in either normotensive or hypertensive animals. These authors propose that there may be possible therapeutic potential for κ-opioid receptor agonists in hypertension. When the acute central actions were examined by microinjection into the hippocampus (Shen & Ingenito, 1999a, 1999b), neither μ- nor δ-opioid receptor agonists produced a reduction in either blood pressure or heart rate to same degree as the non-peptide κ-opioid receptor agonist U-62,066E. These studies suggest that the hippocampal κ-opioid receptor system may contribute to a greater degree than either the μ or δ systems in the central regulation of cardiovascular blood pressure mechanisms.

The latest molecular advances implicating opioid receptor modulation of blood pressure make use of antisense oligodeoxynucleotide (ODN) technology. Antisense ODNs are small DNA fragments that are constructed to be complementary to a defined nucleotide sequence of a specific mRNA (Matteucci & Wagner, 1996; Adams et al., 1994; Chien, C. C. et al., 1994). Recently, these molecular agents have been used to investigate the involvement of opioids in blood pressure regulation. Wright et al. (1999) used an antisense phosphorothionate ODN directed to the N-terminal region of the rat κ-opioid receptor (see Fig. 2) in order to investigate the involvement of this opioid receptor in blood pressure. In normotensive rats, the bi-hippocampal microinjection of the antisense ODN produced hypertension, while in spontaneously hypertensive rats, injection of the same antisense ODN also elevated blood pressure in these already hypertensive animals. Interestingly, while the antisense ODN elevated blood pressure in all animals it was administered to, it did not change the heart rate. This study is the first to use highly sophisticated pharmacological techniques to dissect the κ-opioid receptor from the hippocampus, at the molecu-

lar level, and to allow for an examination of the changes in cellular physiology that result from the absence of the receptor from the tissue. This suggests that a deficiency of the κ-opioid receptor may cause hypertension, and that this receptor may be a CNS component involved in the regulation of blood pressure.

Another mechanism by which hypertension can be reduced involves a reduction in vascular blood volume. This normally can be achieved through the use of diuretic agents such as hydrochlorthiazide (hydroDIURIL®). A diuretic response that results from activation of κ-opioid receptors is well known to occur in many species, including humans (Von Voigtlander & Lewis, 1982b; Peters et al., 1987). While this may not be considered a cardiovascular response, it may be a beneficial 'side effect' in the hypertensive condition.

The diuretic activity of potent κ agonists, such as bremazocine, U-50,488H, U-62,066E, and others, has been the focus of many studies (Huidobro-Toro & Parada, 1985; Leander et al., 1986; Oiso et al., 1988; Yamada et al., 1989, 1990; Bianchi, 1991). All κ agonists studied to date show diuretic properties in many different species, including humans (Von Voigtlander et al., 1981; Von Voigtlander & Lewis, 1982a, 1982b; Peters et al., 1987; Rimoy et al., 1991). The diuresis that results can be blocked by opioid antagonists, including naloxone and MR2266 (Huidobro-Toro & Parada, 1985; Yamada et al., 1989). The endogenous κ-opioid peptide dynorphin A and its related peptide dynorphin $A_{(1-8)}$ have been found to be localized in arginine vasopressin (AVP)-containing neurons of the rat hypothalamic–pituitary–adrenocortical axis (Watson et al., 1982). Although the mechanism is not understood, it is thought that these endogenous κ-opioid peptides modulate the release of AVP from the posterior lobe of the pituitary gland. Leander et al. (1986) and Yamada et al. (1989) showed that U-50,488H and U-62,066E do not produce diuresis in rats that genetically lack AVP. This information led to the suggestion that κ agonists either suppress the neural secretion of AVP or inhibit the effect of AVP on the kidney (Leander et al., 1986). In humans, κ agonists induce water diuresis, without changing renal blood flow or suppressing of AVP levels (Rimoy et al., 1991).

In addition to the effects on AVP levels, many studies suggest that the κ agonists influence the serum levels of other circulating hormones. Iyengar et al. (1985, 1986) showed that U-50,488H and EKC elevated plasma corticosterone, but decreased plasma thyroid stimulating hormone, levels in rats. Thus, although many studies show that the diuretic actions of κ agonists are likely to be due to a κ-opioid receptor effect, clear delineation of the mechanism of action is only now being attempted. This suggests a complex involvement of the opioid system in the regulation of the hypothalamic–pituitary–adrenocortical axis.

Thus, although the exact mechanisms by which opioid receptors modulate the factor(s) responsible for high blood pressure are not known. An examination of the putative mechanisms and efficacy of specific κ-opioid receptor agonists in this disease may provide a novel approach for the development of therapeutically useful antihypertensive drugs. Currently, there is no incentive to pursue this area of research for drug development because of the many adverse events associated with present day κ-opioid agonist drugs.

6.3. The role of peptide and non-peptide opioids in heart failure

CHF is a syndrome characterized by an overall reduction in cardiac performance whereby cardiac output becomes insufficient to maintain adequate tissue and organ perfusion. The cause(s) of heart failure may be clear in some patients (valvular damage, damage to myocardial tissue resulting from ischaemia or infarction, hypertension, or cardiomyopathy), yet may not be clear in others. The usual treatment therapies for CHF includes dietary restriction of salt, thiazide diuretics (such as hydrochlorthiazide), the use of inotropic agents such as cardiac glycosides (digoxin), vasodilator drugs such as captopril, and the administration of opioid analgesics such as morphine.

Many studies suggest that EOPs mediate a depression of myocardial function in CHF states (Holaday, 1983; Barron et al., 1992; Fontana et al., 1993; Llobel & Laorden, 1997). Clinically, elevated levels of EOPs (β-endorphin, met-enkephalin, and dynorphin) have been found in CHF patients, and these may correlate with the severity (Fontana et al., 1993). Naloxone administration to these CHF patients increased blood pressure and heart rate, suggesting a homeostatic regulatory role for EOPs in CHF. However, not all clinical studies suggest that inhibition of opioid peptides is of benefit to patients with acute and chronic heart failure (Lowe, 1991). Oldroyd et al. (1995) found that plasma levels of β-endorphin were normal in patients with acute and chronic heart failure, and did not correlate with the severity of heart failure observed in this study. They also found that naloxone administration did not alter cardiopulmonary exercise performance in these patients, and suggest that EOP inhibition is not likely to have any therapeutic potential (Oldroyd et al., 1995).

Initial research in this area was performed by Liang et al. (1987), who examined the actions of nalmefene, a non-specific opioid receptor antagonist, in a dog model of right ventricular heart failure. In this study, the administration of nalmefene to CHF animals resulted in an increase in aortic blood pressure, cardiac output, left ventricular function (dP/dt), blood flow to the myocardium, and plasma β-endorphin levels. Many additional studies using nalmefene and naloxone in CHF models also suggest that blockade of opioid receptors is beneficial (Himura et al., 1994; Sakamoto et al., 1989). It is suggested that the improvement in haemodynamic and cardiac function results from inhibition of opioid receptors in the CNS, since the use of naloxone methylbromide, a charged derivative of naloxone that cannot cross the blood–brain barrier, did not produce these beneficial actions. Thus, while the use of opioid receptor antag-

onists suggests that opioid receptors may be involved in heart failure, these agents do not provide information with regard to which opioid receptor subtype may be specifically involved or the mechanism by which blockade of opioid receptors is of benefit to the failing myocardium. However, in an attempt to investigate which opioid receptor subtype may be involved in CHF, and since naloxone was shown to be of benefit in CHF and is known to block both the μ- and δ-opioid receptor subtypes, Imai et al (1994) studied the relative role of these opioid receptors in CHF. The selective δ-opioid receptor antagonist ICI-154,129 and the selective μ receptor antagonist naloxonazine were used. In a manner similar to naloxone, only the δ receptor antagonist improved cardiac performance, systemic haemodynamics, and regional blood flow in animals with CHF (Imai et al., 1994). Thus, this study suggests that blockade of δ-opioid receptors during CHF is beneficial since it prevents the reduction in contractility associated with CHF. However, the role and mechanism of action of EOPs remains entirely speculative, based on the current studies that have been conducted. The exact mechanism(s) by which non-peptide opioid agonists such as morphine produce beneficial effects in CHF is not known. While morphine produces bradycardia and hypotension by a decrease in peripheral resistance (Greenberg et al., 1994), it is suggested that at clinical doses (0.2 mg/kg), the effects of morphine on the CNS are more important (Timmis et al., 1980). Morphine reduces pain, as well as anxiety and dyspnoea, that may be associated with pulmonary edema-related complications in CHF (Greenberg et al., 1994).

Thus, while research suggests that a beneficial effect may be achieved with opioid receptor antagonists in CHF, these studies merely implicate EOPs, a relatively unknown causative factor, to this syndrome. No detailed mechanism has been provided yet to explain the weak temporary effects of these opioid receptor antagonists in heart failure and the role of EOPs in the underlying deterioration of heart function associated with this syndrome.

6.4. Peptide and non-peptide opioid involvement in ischaemic preconditioning

Myocardial ischaemic preconditioning (PC) is a phenomenon that occurs in cardiac muscle in which brief periods of ischaemia (usually less than 5 min in duration) render the muscle tolerant to tissue damage that occurs during a subsequent period of ischaemia, following an interlude of reperfusion (Murry et al., 1986) (Fig. 5A). Such a phenomenon has been shown to occur in many species, and is known to be mediated by a well-defined intracellular cascade (Dana & Yellon, 1998). However, the intercellular mediator or PC-induction trigger appears to be diverse, and has been shown to involve opioids and many other substances (for reviews, see Rubino & Yellon, 2000; Dickson et al., 2001). Thus, the nature of ischaemic PC may not be consistent between the species examined, despite a common end result. This may have significant implications for humans, both in terms of the use of animal models to elucidate the cellular mechanisms involved in cardiac muscle protection, as well as the development of novel therapeutic agents useful in ischaemic infarction. Regardless, molecular pharmacological techniques are being employed to explore this phenomenon in myocardial dysfunction and to attempt to elucidate the mechanism(s) by which opioid receptors are implicated.

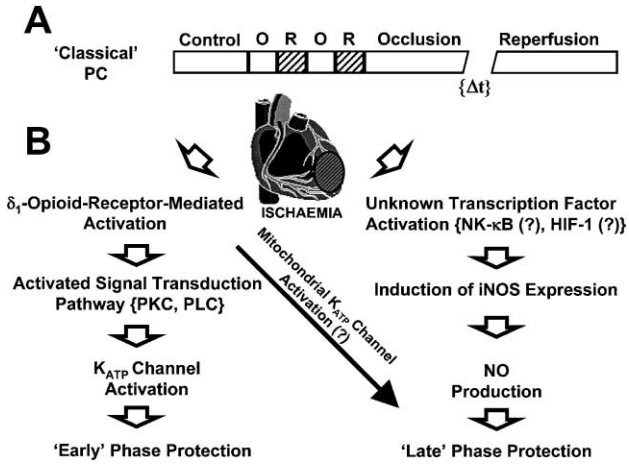

Fig. 5. **A**: A "typical" protocol bar that may be used in experiments that investigate the effects of agents on ischaemic PC in the heart. Similar protocols have been used to study opioid receptor-mediated cardioprotective mechanisms in the rat and other mammalian species. Studies may investigate the effects of ischaemic PC on ischaemia and/or reperfusion arrhythmias of various durations in length (Δt). Ischaemic PC usually involves a sequence of coronary artery occlusion (O), followed by reperfusion (R) cycles. In this panel, two cycles are depicted prior to coronary artery occlusion followed by reperfusion. *Note that this type of protocol may be easily amenable to modification, the protocol shown is a simplified protocol bar.* **B**: Schematic diagram that describes the molecular pathways involved in the protective effects of ischaemic PC in the heart. Brief periods of ischaemia provide both an 'early' and 'late' phase of protection to the heart from subsequent episodes of prolonged or permanent ischaemia. The activation of G-protein-coupled δ_1-opioid receptors has been shown to be involved in both protective phases. The activation of the δ_1-opioid receptor activates intracellular signal transduction pathways [including PKC, phospholipase C (PLC), and related kinase pathways]. Activation of these pathways results in the phosphorylation of certain proteins such as the K_{ATP} channel present both on the myocyte cell surface, as well as the myocyte mitochondrial cell surface. The opening of these ion channels mediates the 'early' phase of cardioprotection that lasts 2–3 hr after the ischaemic episode. The 'late' phase of cardioprotection that results from the brief episodes of ischaemia may be mediated by the activation of oxygen-sensitive transcription factors (such as NF-κB and HIF-1). These factors subsequently induce the expression of inducible NOS (iNOS) and the production of NO, the putative mediator of the protective effect on the heart observed 24–48 hr after the ischaemic episode (Guo et al., 1999). Recently, it has been shown that δ_1-opioid receptor stimulation may also produce a 'late' cardioprotective effect that may result from activation of mitochondrial K_{ATP} channels (see Fryer et al., 1999 for details).

In vivo studies show that ischaemic PC can reduce the size of an infarction resulting from prolonged ischaemia (Gross & Fryer, 1999). There is also a reduction in damage to myocardial intracellular structures, a decrease in the dysfunction of the cardiac contractile machinery, and a direct

reduction in arrhythmias associated with ischaemic PC (Light, 1999; Gross & Fryer, 1999). The ability of ischaemic PC to limit myocardial damage occurs chronologically in two distinct phases. The first, or early phase, provides a window of protection to the heart muscle that occurs soon after PC and declines with time during the first 3 hr of reperfusion. The second, late (or delayed) phase of PC, provides a second window of protection to heart muscle that emerges after $\sim$ 24 hr of reperfusion (Bolli et al., 1998; Guo et al., 1998; Bolli & Marban, 1999). The biphasic nature of this phenomenon has given rise to the individual study of each phase, and has resulted in the development of a list of putative mediators that are primarily thought to be responsible for this protective action in the heart. The development of selective pharmacological agents that either activate or mimic this mediator could significantly advance our understanding of myocardial ischaemia, while characterization of the mediator may aid in the development of a novel pharmacological target with a marked therapeutic potential. Many potential mediators have been implicated in PC, these include nitric oxide (NO), adenosine, bradykinin, ATP-dependent K^+ (K_{ATP}) channels, prostacyclin, oxygen-sensitive transcriptional factors such as nuclear factor-κB (NF-κB), and hypoxia-inducible factor (HIF)-1, as well as various enzymes, kinases, G-proteins, ion channels, and opioid agonists (Gross & Fryer, 1999; Rubino & Yellon, 2000). While the list seems to be composed of a diverse group of mediators, studies in cardiac muscle have more completely characterized those involved in the early phase of protection. Only recently has attention focussed on the mechanism(s) involved in the delayed phase of protection.

The early phase of ischaemic PC is an immediate response mechanism initiated by the myocardial cell, and involves the activation of G-protein-coupled membrane receptors. Studies suggest that while bradykinin receptors may be involved, adenosine may be a predominant mediator of this early response (Lowenstein, 1999). However, it is well recognized both functionally and in terms of molecular architecture that opioid receptors belong to this family of G-protein-coupled receptors (Schultz et al., 1998b).

Direct opioid receptor activation, in a manner similar to related G-protein-coupled receptors, can result in tissue-specific inhibitory (Konkoy & Childers, 1989) or stimulatory actions on several intracellular second messenger systems. The mechanism by which opioid receptor-mediated transduction signaling occurs is not well understood and beyond any in-depth description in this review. However, many studies suggest that opioid receptor activation stimulates adenylate cyclase and the formation of cyclic AMP, stimulates phosphoinositide hydrolysis, elevates intracellular Ca^{2+} levels by enhancing either Ca^{2+} influx into cells or Ca^{2+} release from intracellular stores activates protein kinase C (PKC) and protein kinase A (PKA). and may modulate both voltage-gated Na^+ and K^+ channels (Periyasamy & Hoss, 1990; Childers, 1991; for a review, see Smart & Lambert, 1996).

In ischaemic PC, recognition of the effector molecule (adenosine, bradykinin, opioids) results in activation of the coupling G-protein and possibly several intracellular pathways. The ultimate action of these intracellular messengers is thought to involve the phosphorylation of an end-effector molecule. Many studies suggest that this end-effector molecule may be the K_{ATP} channel (Gross & Fryer, 1999). While the K_{ATP} channel has been found in many tissues, including the pancreas, kidneys, and brain, it has been shown to play a significant role as an endogenous cardioprotective ion channel in the heart under conditions of myocardial ischaemia (Gross & Auchampach, 1992). The mechanism by which the activation of the K_{ATP} channel is cardioprotective is not known. It is suggested that these channels maintain cellular osmolarity (Light, 1999), in addition to their ability to shorten the APD and to protect the cell from intracellular Ca^{2+} overload during ischaemia (Cole et al., 1991). The K_{ATP} channel is found ubiquitously throughout the heart, and is regulated by the ratio of ADP to ATP, as well as by PKA and PKC. Recently, the plasma membrane isoform of this channel has been cloned and functionally expressed (for a review, see Light, 1999). The cloned K_{ATP} channel is a complex that consists of a pore-forming, inwardly rectifying subunit (Kir 6.2) and a sulfonylurea subunit (SUR2A). The co-expression of these subunits results in a channel that has similar properties to the native channel found in the heart. The molecular constituents of the mitochondrial isoform of the K_{ATP} channel are not known presently. Pharmacologically, however, the plasma membrane and mitochondrial isoforms of the channel are distinct. While both isoforms of the channel have similar sensitivities to K_{ATP} channel openers, such as pinacidil and cromakalim, the mitochondrial isoform is significantly more sensitive to diazoxide, an unrelated channel opener (Garlid et al., 1997). It is the opening of the K_{ATP} channel that is considered to be a pivotal component in the protective effects exhibited by opioid receptor activation during the early phase of ischaemic PC. The involvement of the K_{ATP} channel in the late phase of ischaemic PC has also been suggested, but studies implicate another mediator in this phase, NO.

Unlike the controversial involvement of NO as a mediator of the early phase of PC, the late phase of ischaemic PC clearly has been shown to closely involve NO synthase (NOS) activity (Bolli et al., 1997; Takano et al., 1998). Three gene products result in the enzymes that synthesize NO. These gene products include the constitutive production of NO by endothelial NOS and neuronal NOS enzyme isoforms, as well as an inducible (iNOS) enzyme isoform (Kelly et al., 1996). The involvement of NO in the late phase of PC derives from in vivo studies where the cardioprotective effects associated with the late phase can be abolished by selective iNOS inhibitors, such as aminoguanidine or S-methylisothiourea sulfate (Bolli et al., 1997). In ischaemic PC, iNOS expression has been shown to be regulated by several transcriptional factors, including

NF-κB (Xuan et al., 1999) and HIF-1 (Palmer et al., 1998). Only recently has Guo et al. (1999) been able to show conclusively that mice that lack the iNOS gene (iNOS $^{-/-}$) do not exhibit the late phase of ischaemic PC cardioprotection, yet these animals retain the cardioprotection associated with the early phase of ischaemic PC. Thus, while both the early and late phases of ischaemic PC are suggested to be mediated by distinctly independent mechanisms (see Fig. 5B for details), some studies suggest that opioids, via activation of mitochondrial K_{ATP} channels, may produce a delayed cardioprotective effect (Fryer et al., 1999).

The involvement of opioids in ischaemic PC resulted from a recognition of their value at increasing survival time and tissue preservation prior to surgical transplantation and of their possible role in enhancing tolerance to hypoxia (Mayfield & D'Alecy, 1992; Chien, S. et al., 1994). Schultz et al. (1995) were the first to propose that opioid receptor activation may be involved in ischaemic PC in rat myocardial tissue. Naloxone abolished PC-mediated cardioprotection, suggesting that PC may involve endogenous opioid agonist activity. Prior to these studies, it had been shown that opioid receptors were linked, by G-proteins, to ion channels (Chen & Yu, 1994; North et al., 1987) and that blockade of myocardial ATP channels, using the K_{ATP} inhibitors glibenclamide and 5-hydroxydecanoate, inhibited ischaemic PC (Gross & Auchampach, 1992; Auchampach et al., 1992). Subsequent studies by Schultz et al. (1996) suggested that morphine-mediated opioid receptor stimulation reduced the size of infarcts in rat hearts in a manner similar to that produced by ischaemic PC alone. In their study, both glibenclamide and naloxone blocked the cardioprotective action of morphine, suggesting that opioids mediate ischaemic PC via activation of K_{ATP} channels. Subsequently, naloxone blockade of PC-mediated cardioprotection was shown to be stereospecific, and since naloxone blocked PC in isolated hearts, the effect was clearly not mediated by the CNS (Schultz et al., 1997a; Chien & Van Winkle, 1996; Chien et al., 1999). Confirmation of the involvement of K_{ATP} channels in ischaemic PC has been established using many animal models and many drugs, including K_{ATP} channel openers and blockers (Gross & Fryer, 1999).

Delineation of the opioid receptor subtype involved in ischaemic PC was characterized using selective opioid receptor agonists and antagonists. The first studies of this nature were conducted by Mayfield and D'Alecy (1994a, 1994b). These studies were the first to pharmacologically characterize the acute protective effect of opioid receptor activation arising from stressful situations associated with hypoxic or cold environmental conditions. These studies suggested that the mechanism of adaptation was sensitive to blockade of the δ_1-opioid receptor (Mayfield & D'Alecy, 1994a). To determine whether the δ-opioid receptor played a similar role in ischaemic PC in the heart, Schultz et al. (1997b) used naltrindole, a δ-opioid receptor antagonist, and found that this antagonist abolished myocardial protection and attenuated infarct size reduction by ischaemic PC and morphine. Subsequent studies examined the role of μ- and κ-opioid receptors in this phenomenon. Nor-BNI, a κ-opioid receptor antagonist, and β-funaltrexamine, an irreversible μ-opioid receptor antagonist, were without effect in rat hearts, strongly suggesting that the δ-opioid receptor in the myocardium alone mediates a protective role in ischaemic PC (Schultz et al., 1997b; Tsuchida et al., 1998). It is recognized that at least two subtypes of the δ-opioid receptor, δ_1 and δ_2, can be pharmacologically characterized in the CNS (Mattia et al., 1991), and that δ-opioid receptors exist in the heart (Krumins et al., 1985; Ventura et al., 1989). The use of selective δ-opioid receptor antagonists, such as 7-benzylidenenaltrexone (BNTX), a selective δ_1-opioid receptor antagonist, or naltriben, a selective δ_2-opioid receptor antagonist, prior to ischaemic PC in rats has helped to delineate the δ-opioid receptor subtype involved in cardioprotection. Only BNTX significantly attenuated the cardioprotective effect of ischaemic PC, indicating that, as was shown by Mayfield and D'Alecy (1994a), that the δ_1-opioid receptor plays a crucial role in protective cellular responses to hypoxia and ischaemia (Schultz et al., 1998a).

Until recently, as mentioned earlier in this section, opioid receptors were thought to elicit only an early phase, or first window, of cardioprotection. However, Fryer et al. (1999) recently completed a study that examined the role of the δ_1-opioid receptor subtype in the late phase, or second window, of cardioprotection. The δ_1-opioid agonist 2-methyl-4aα-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12aα-octahydroquinolino[2,3,3-g]isoquinoline chloride (TAN-67) significantly decreased the ratio of myocardial infarct size/area at risk when administered between 24 and 72 hr before the onset of ischaemia and reperfusion. The TAN-67-induced cardioprotection was abolished by the administration of the selective δ_1-opioid receptor antagonist BNTX. Mechanistically, the cardioprotection that was conferred by activation of the δ_1-opioid receptor was prevented by glibenclamide, a K_{ATP} blocker, and 5-hydroxydecanoate, a mitochondrial K_{ATP}-channel blocking drug (Fryer et al., 1999). Activation of these ion channels suggests that in cardiac muscle, the delayed component, in addition to up-regulation of NOS, may result from the opening of mitochondrial K_{ATP} channels (Fryer et al., 1999). These observations can also be reproduced in isolated cardiac myocytes under conditions that simulate ischaemia (Liang & Gross, 1999).

Thus, although these studies conclude that specific cardiac ion channels are involved in both the first and second windows of cardioprotection resulting from ischaemic PC, future studies must be conducted with more selective agonist and antagonist drugs for the K_{ATP} channel present on the cell membrane, as well as the mitochondria. Despite this realization, experimental evidence strongly suggests that the δ_1-opioid receptor pharmacologically modulates cardiovascular processes involved in ischaemic PC that may be of value in the study of mechanisms or the development of therapies for the treatment of myocardial infarction in patients.

6.5. The actions of peptide and non-peptide opioids in ischaemic arrhythmogenesis

The role of opioid receptors in ischaemic arrhythmogenesis remains a contentious issue. The involvement of opioid receptors in ischaemic arrhythmias has been inferred principally from studies that use various selective and non-selective blockers of these receptors. The problem that has developed from these studies relates to the inconsistency of data that defines the role of opioid receptors as mediating pro-arrhythmic or antiarrhythmic actions in cardiac muscle (Curtis et al., 1993). It has been reported that both opioid receptor agonists, as well as antagonists, reduce arrhythmia incidence resulting from myocardial ischaemia.

Many studies suggest that blockade of the κ-opioid receptor may be antiarrhythmic (Sitsapesan & Parratt, 1989; for a review, see Pugsley et al., 1993a). The first reported effects of the involvement of an opioid receptor agonist drug in ischaemic arrhythmias were made by Fagbemi et al. (1982). Prior to this study, it had only been observed that opioids were of benefit in shock states (Holaday & Faden, 1978). Naloxone, when given at doses similar to those used in shock states, reduced the incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) in rats subject to coronary artery occlusion (Fagbemi et al., 1982). It was postulated in these early studies that EOPs such as cardiac β-endorphin may be endogenous arrhythmogenic components released into the local cellular milieu during the development of myocardial ischaemia, although there was no direct evidence to substantiate this action. Thus, these components could exhibit detrimental electrophysiological effects on the myocardium either by directly interacting with local opioid receptors or by modulating the ion channel components of the myocardium and, hence, alter cardiac action potentials (Fagbemi et al., 1982).

It is well known that opioid receptor actions in the CNS are stereospecific. Thus, the involvement of an opioid receptor, the subtype that has yet to be fully delineated, in arrhythmogenesis has been examined using the stereoisomers of different antagonists at opioid receptors (Parratt & Sitsapesan, 1986). The use of (−)Mr1452 and (−)WIN 44,441-3, both stereoselective κ-opioid receptor antagonists, dose dependently decreased the incidence of ischaemic arrhythmias in rats, suggesting that blockade of κ-opioid receptors in the myocardium is antiarrhythmic. The inactive opioid receptor antagonist isomers of these drugs, (+)Mr1453 and (+)WIN44,441-2, were inactive. Mackenzie et al. (1986) and Sitsapesan and Parratt (1989) then showed that naloxone, at doses that inhibit μ- and κ-opioid receptors, and the selective κ antagonist MR2266 reduced ischaemic arrhythmias in rats. The quaternary naloxone derivative MrZ 2593 was also effective against ischaemic arrhythmias, and since it did not cross the blood–brain barrier, suggested that the antiarrhythmic actions of opioids may be mediated by peripheral opioid receptors (Sitsapesan & Parratt, 1989). Recently, Murphy and Murphy (1999) showed that peripheral opioid receptor blockade by methylnaltrexone, a quaternary derivative of naltrexone that does not cross the blood–brain barrier, also protected the heart against ischaemia-induced arrhythmias.

The majority of the studies published to date suggest that there is an involvement of EOPs in ischaemic arrhythmias, as opposed to the effects of opioid receptor agonist and antagonist drugs (for a review, see Lee, 1990). Many studies confirm the antiarrhythmic actions of opioid receptor antagonists, such as naloxone against arrhythmias produced by many methods, including chloroform-hypoxia in rats (Wong & Lee, 1985), and ischaemia in isolated rat (Zhan et al., 1985) and canine hearts (Huang et al., 1986; Lee, 1992). Lee et al. (1992) showed that the cardiac κ-opioid receptor may be involved in ischaemic arrhythmias. In their studies, naloxone attenuated ischaemic arrhythmias produced by the κ-opioid receptor agonist U-50,488H. These studies suggested, as originally proposed by Sitsapesan and Parratt in 1989, that the cardiac κ-opioid receptor may be the opioid receptor subtype that is involved in ischaemic arrhythmogenesis. These results are in agreement with those published by Pugsley et al. (1992a), who showed that the κ-opioid receptor agonist U-50,488H, at low doses, has no antiarrhythmic action, but at higher doses, *reduces* the incidence of ischaemic arrhythmias (see Fig. 6 for the antiarrhythmic actions of U-50,488H and related opioid drugs). These results are in agreement with the dual action that κ-opioid receptor agonists may have in ischaemic arrhythmias. Kaschube and Brasch (1991) and Pugsley et al. (1992a, 1993b) suggested that the potentially arrhythmogenic actions of κ-opioids occur at low doses, due to activation of the opioid receptor, and that antiarrhythmic actions occur at higher doses, due to a non-opioid receptor, direct interaction with the cardiac membrane (see Section 6.2 for a discussion of the ion channel blocking actions of κ-opioid receptor drugs).

The opioid receptor-independent actions of opioid compounds was observed by Fagbemi et al. (1983), who showed, using the opioid receptor partial agonist, meptazinol, that opioids directly interact with the cardiac ion channel. Studies by Sagy et al. (1987) concurred, showing that the opioid antagonists naloxone and d-naloxone (the stereoisomer that is inactive as an opioid receptor antagonist) exert a direct local effect (positive inotropism) on isolated hearts and in isolated and spontaneously contracting atrial tissue preparations. These direct inotropic actions were observed in paced human atrial preparations (Sagy et al., 1987). Consistent with these findings, Boachie-Ansah et al. (1989) found that buprenorphine, an agent having mixed μ agonist and κ antagonist properties, depressed the V_{max} and increased the APD in heart tissue due to Na^+-channel blockade and delayed-outward K^+-channel blockade, respectively. This study concluded that the antiarrhythmic actions of this mixed μ agonist and κ antagonist drug result independently of actions on opioid receptors. Buprenorphine saturation of either the μ- or κ-opioid receptor would occur at a dose of 250–500 μg/kg. However, the doses of buprenorphine that

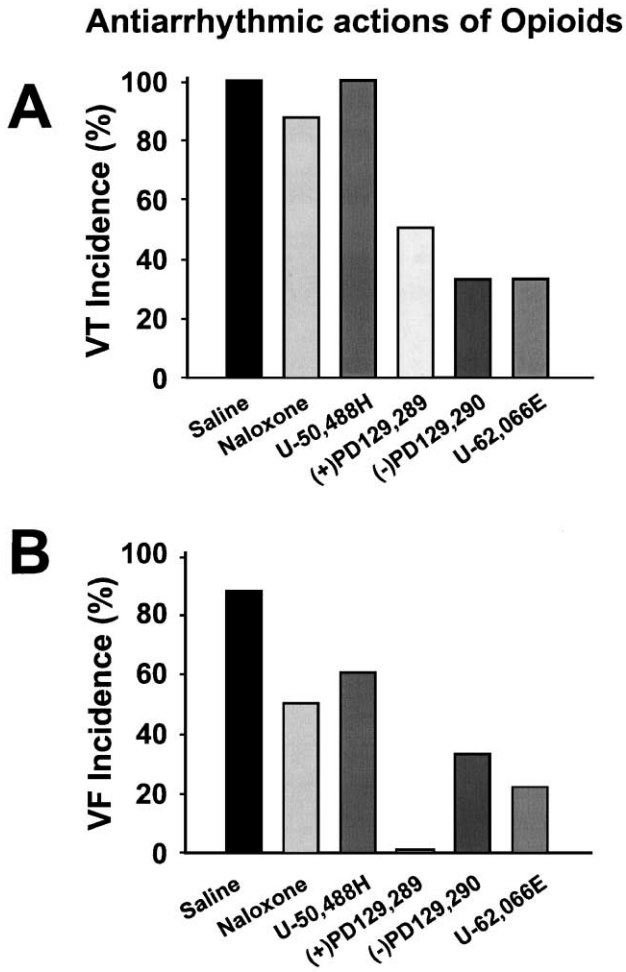

Fig. 6. A vertical bar chart that describes the antiarrhythmic actions of a series of arylacetamide κ-opioid drugs and the opioid receptor antagonist naloxone on the incidence of arrhythmias that result from occlusion of the left-anterior descending coronary artery in pentobarbitone-anesthetized, artificially ventilated rats. The antiarrhythmic actions are expressed as the percent of animals that experience one or more episodes of the major arrhythmias of VT or VF for 30 min post-occlusion. **A**: the incidence of VT for each drug-treated group. **B**: the incidence of VF for each drug-treated group. Drugs were administered as an intravenous bolus dose 2 min prior to coronary artery occlusion. The drugs administered were saline (0.3 mL, $n = 15$), naloxone (8.0 μmol/kg, $n = 15$), U-50,488H (16 μmol/kg, $n = 15$), (+)PD129,289 (8.0 μmol/kg, $n = 9$), (−)PD129,290 (8.0 μmol/kg, $n = 9$), and U-62,066E (2.5 μmol/kg, $n = 15$).

were administered to suppress arrhythmias (1.0 mg/kg), or concentrations that depress the V_{max} or prolong the APD, would result in saturated opioid receptors and, thus, tend to eliminate the possible involvement of specific opioid receptor activation (Boachie-Ansah et al., 1989). Similarly, Sarne et al. (1991) found that the incidence of ischaemic arrhythmia could be reduced by the opioid receptor antagonists naloxone and Win 44,441-3, as well as their respective non-opioid stereoisomers (+)naloxone and Win 44,441-2. The opioid receptor agonists levorphanol and morphine and the (+)stereoisomer dextrorphan were all antiarrhythmic, suggesting that opioid receptor agonist and antagonist drugs may mediate these actions independent of opioid receptors. This suggested opioid receptor-independent mechanism confirms earlier findings of the Na^+-channel blocking actions of morphine in giant squid axons by Frazier et al. (1973) and the blockade of time-dependent outward K^+ currents in guinea pig isolated atria by the optical isomers (+)- and (−)-naloxone (Brasch, 1986).

While many studies suggest either a pro- or antiarrhythmic role for opioid agonists and antagonists in ischaemic arrhythmias, additional studies complicate the issue by finding a lack of effect of opioid drugs in cardiac tissue. Bergey and Beil (1983) found that naloxone, at doses up to 10 mg/kg, failed to protect against arrhythmias and death following acute coronary artery occlusion in pigs. The administration of the opioid antagonist at this dose results in tissue and blood concentrations that are in the order of 50–100 times the dose required for the antagonism of exogenously applied agonists for opioid receptors in vivo (Martin, 1984). When similar high concentrations of naloxone were examined electrophysiologically (2–10 μM), they produced little or no change in the action potential configuration (Pruett et al., 1991).

Recently, the use of selective arylacetamide κ-opioid receptor agonists, such as U-50,588H and U-62,066E, has made it possible to establish the involvement of the κ-opioid receptor in sedation, analgesia, and diuresis, but has also made it possible to dissociate the involvement of this opioid receptor in cardiac arrhythmogenesis (Pugsley et al., 1993a).

U-50,488H, a selective κ receptor agonist, has been shown to induce ventricular arrhythmias in isolated perfused rat hearts, when given as a bolus of 44 or 132 nmol (Wong et al., 1990). The incidence and severity of ischaemia-induced arrhythmias produced by U-50,488H are reduced by the selective κ-opioid receptor antagonist MR2266 (Wong et al., 1990). From these results, these authors suggest that there may be a role for the κ-opioid receptor in the genesis of arrhythmias during ischaemia (and reperfusion). This can only be so provided that there is an endogenous κ-opioid receptor agonist that is localized in *ischaemic* tissue. Immunological studies show that the peptide dynorphin, an endogenous κ-opioid receptor agonist, is present in the heart (Weihe et al., 1985). However, no studies have been conducted that show that these κ-opioid receptor peptides are elevated under ischaemic conditions. However, in contrast to these studies, U-50,488H and several other arylacetamide κ agonists, such as PD129,290, (−)CI 977, and U-62,066E (spiradoline), have been shown to reduce the severity of ischaemic and electrically induced arrhythmias in rats (Pugsley et al., 1992a, 1992b, 1993b, 1998). Pugsley et al. (1992a, 1993b, 1998) have shown that these arylacetamide compounds retain their antiarrhythmic actions in vivo in the presence of opioid receptor blockade. Subsequent electrophysiological studies conducted in isolated rat ventricular myocytes (for an example, see Fig. 7), in the absence and presence of naloxone, showed unequivocally that these arylacetamide compounds produce direct blockade of car-

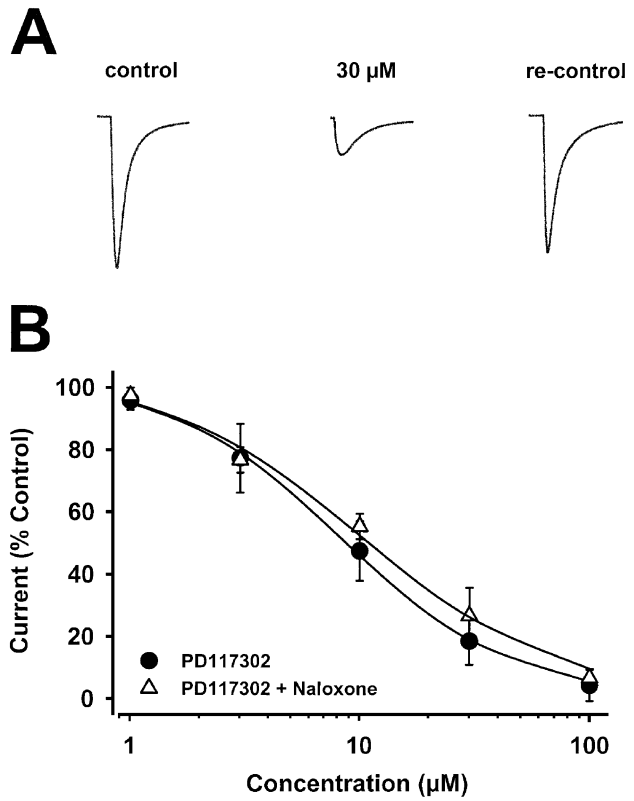

Fig. 7. Voltage-dependent block of the transient Na^+ current by the κ-opioid receptor agonist PD117,302 is concentration-dependent in rat cardiac myocytes. **A**: Na^+ currents traces evoked by a voltage step from a pre-pulse potential of − 130 mV to a potential of 0 mV. The voltage step was delivered at 6-sec intervals and PD117,302 was added to the bath solution at the concentration indicated in **B**. A 30-μM concentration of PD117,302 produced marked blockade of the Na^+ current. However, the recontrol current is indistinguishable from the control. PD117,302 was added to the bath solution for 120 sec before evoking currents at any of the concentrations examined. **B**: concentration–response curve for the degree of blockade of the transient Na^+ current by PD117,302 in the absence and presence of 1.0-μM naloxone. Na^+ currents were evoked as above. The degree of block, $I_{(control)} - I_{(block)}/I_{(control)}$, is shown as a function of the $\log_{10}$ concentration of PD117,302. Data from five individual cells is plotted, with the line of best fit for the equation $y = 1/(1 + (Ka/[A]))$ shown. The estimated K_a from this equation is 10 ± 3 μM for PD117,302.

diac Na^+ and K^+ ion channels (Pugsley et al., 1994, 1998). A recent structure–activity relationship analysis of 10 arylacetamide drugs provides additional evidence that suggests that the antiarrhythmic actions of these drugs reside in their ability to block both cardiac Na^+ and K^+ currents, and is not related to κ-opioid receptor agonism (Yong et al., 1996). Pugsley and Goldin (1999) demonstrated that RSD 921, a (+)-enantiomer of a κ-opioid receptor agonist and an aryl derivative of benzacetamide currently under investigation as an antiarrhythmic drug, blocks the open state of cardiac Na^+ channels expressed in *X. laevis* oocytes. This study suggests, for the first time, that arylacetamide drugs that lack κ-opioid receptor agonist actions may be promising new candidates for the development of novel drugs for the treatment of cardiac arrhythmias associated with myocardial ischaemia.

The arylacetamides were developed as structurally novel agonists for the κ-opioid receptor. However, these compounds, when administered exogenously, exhibit opioid receptor-independent properties in cardiac tissue. It is this property of these compounds that makes them valuable in the study of arrhythmogenic mechanisms, useful in the possible treatment of ischaemic arrhythmias, or for use as tools in the study of cardiac ion channel function. The lack of structural similarity of the arylacetamides to either Class I (Na^+-channel blocking) or Class III (K^+-channel blocking) antiarrhythmic agents and dissociation of κ-opioid receptor agonist properties (and inherent adverse events associated with κ-opioid receptor agonism) offers an opportunity to develop and to understand the different structural requirements for drug effectiveness in ischaemic arrhythmogenesis.

7. Conclusion

The cardiovascular profile of action of opioid receptor agonists and antagonists is not complete. This review showed that these drugs can mediate actions both centrally and peripherally by either opioid receptor-dependent or opioid receptor-independent mechanisms. While it remains difficult to fully explore the role opioid receptors have in the cardiovascular system, many studies have been conducted using antagonist and opioid receptor stereoisomers that lack opioid receptor activity. Attempts are being made to investigate opioid receptor involvement in certain cardiovascular pathophysiological states, such as heart failure and hypertension. The inherent degree to which opioid receptors are involved in these various disease states requires further investigation. Perhaps opioid receptor involvement in heart and cardiovascular function can now be defined at a molecular level using gene "knock-down" or gene "knock-out" mice.

Conversely, ischaemic arrhythmogenesis may not depend upon participation of the opioid receptor. Studies that involve cardiac tissue present the clearest evidence for these opioid receptor-independent actions. Electrophysiological studies conducted in cardiac preparations suggest that opioid receptor agonists and antagonists exert Na^+ and K^+ ion channel blocking properties. In the heart, these actions are associated with antiarrhythmic effects during ischaemia-induced arrhythmias that result from coronary artery occlusion.

While the clinical relevance of opioid receptor-mediated actions on the cardiovascular system may be limited to only a few pathophysiological conditions, the efficacy of drugs such as the arylacetamide κ-opioid receptor drugs against ischaemic arrhythmogenesis may provide important details regarding the development of novel antiarrhythmic drugs. Further examination of these compounds may allow for the elucidation of a series of pharmacological structures, devoid of κ-related properties, that possess *selective* Na^+- and K^+-channel blocking properties in cardiac tissue, especially

under ischaemic conditions. This would then allow for the development of novel antiarrhythmic drugs that might prove useful in a clinical setting.

Acknowledgements

I wish to thank the Heart and Stroke Foundation of B.C. and Yukon, Science Council of B.C., and the Medical Research Council of Canada for Graduate and Post-Graduate research support. I am greatly indebted to M. J. A. Walker, D. A. Saint, and A. L. Goldin for their guidance during these studies.

References

Adams, J. U., Chen, X., DeRiel, J. K., Adler, M. W., & Liu-Chen, L. Y. (1994). Intracerebroventricular treatment with an antisense oligodeoxynucleotide to kappa-opioid receptors inhibited kappa-agonist-induced analgesia in rats. *Brain Res 19*, 129–132.

Akil, H., Mayer, D. J., & Liebesking, J. C. (1974). Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. *Science 191*, 961–962.

Alarcón, S., Hernández, J., & Laorden, M. L. (1993). Cardiac electrophysiological effects of U-50,488H on guinea-pig papillary muscle. *Neuropeptides 24*, 313–316.

Altura, B. T., Altura, B. M., & Quirion, R. (1984). Indentification of benzomorphan-κ opiate receptors in cerebral arteries which subserve relaxation. *Br J Pharmacol 82*, 459–466.

Alzheimer, C., & ten Bruggencate, G. T. (1990). Nonopioid actions of the κ-opioid receptor agonists, U-50,488H and U-69,593, on electrophysiologic properties of hippocampal CA3 neurons *in vitro*. *J Pharmacol Exp Ther 255*, 900–905.

Auchampach, J. A., Grover, G. J., & Gross, G. J. (1992). Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. *Cardiovasc Res 26*, 1054–1062.

Barron, B. A. (1999). Opioid peptides and the heart. *Cardiovasc Res 43*, 13–16.

Barron, B. A., Gu, H., Gaugl, J. F., & Caffrey, J. L. (1992). Screening for opioids in dog heart. *J Mol Cell Cardiol 24*, 67–77.

Barron, B. A., Jones, C. E., & Caffrey, J. L. (1995). Pericardial repair depresses canine cardiac catecholamines and met-enkephalin. *Regul Pept 59*, 313–320.

Beckett, A. H., & Casy, A. F. (1954). Synthetic analgesics: stereochemical considerations. *J Pharm Pharmacol 6*, 986–999.

Bergey, J. L., & Beil, M. E. (1983). Antiarrhythmic evaluation of naloxone against acute coronary occlusion-induced arrhythmias in pigs. *Eur J Pharmacol 90*, 427–431.

Bianchi, G. (1991). Antidiuretic effect of bremazocine and U-50,488 in rats after α_2-adrenoceptor blockade. *J Pharm Pharmacol 43*, 212–216.

Boachie-Ansah, G., Sitsapesan, R., Kane, K. A., & Parratt, J. R. (1989). The antiarrhythmic and cardiac electrophysiological effects of buprenorphine. *Br J Pharmacol 97*, 801–808.

Bolli, R., & Marban, E. (1999). Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev 79*, 609–634.

Bolli, R., Bhatti, Z. A., Tang, X. L., Qiu, Y., Zhang, Q., Guo, Y., & Jadoon, A. K. (1997). Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide. *Circ Res 81*, 42–52.

Bolli, R., Dawn, B., Tang, X. L., Qiu, Y., Ping, P., Xuan, Y. T., Jones, W. K., Takano, H., Guo, Y., & Zhang, J. (1998). The nitric oxide hypothesis of late preconditioning. *Basic Res Cardiol 93*, 325–338.

Brasch, H. (1986). Influence of the optical isomers (+)- and (−)-naloxone on beating frequency, contractile force and action potentials of guinea-pig isolated cardiac preparations. *Br J Pharmacol 88*, 733–740.

Caffrey, J. L. (1999). Enkephalin inhibits vagal control of heart rate, contractile force and coronary blood flow in the canine heart in vivo. *J Auton Nerv Syst 76*, 75–82.

Carratu, M. R., & Mitolo-Chieppa, D. (1982). Inhibition of ionic currents in frog node of ranvier treated with naloxone. *Br J Pharmacol 77*, 115–119.

Chang, K. J., Blanchard, S. G., & Cuatrecasas, P. (1984). Benzomorphan sites are ligand recognition sites of putative epsilon-receptors. *Mol Pharmacol 26*, 484–488.

Chen, J. C., Smith, E. R., Cahill, M., Cohen, R., & Fishman, J. B. (1993). Molecular cloning and functional expression of a μ-opioid receptor from rat brain. *Mol Pharmacol 44*, 8–12.

Chen, Y., & Yu, L. (1994). Differential regulation by cAMP-dependent protein kinase and protein kinase C of the mu opioid receptor coupling to a G protein-activated K^+ channel. *J Biol Chem 269*, 7839–7842.

Chien, C. C., Brown, G., Pan, Y. X., & Pasternak, G. W. (1994). Blockade of U50,488H analgesia by antisense oligodeoxynucleotides to a kappa-opioid receptor. *Eur J Pharmacol 253*, R7–R8.

Chien, G. L., & Van Winkle, D. M. (1996). Naloxone blockade of myocardial ischemic preconditioning is stereoselective. *J Mol Cell Cardiol 28*, 1895–1900.

Chien, G. L., Mohtadi, K., Wolff, R. A., & Van Winkle, D. M. (1999). Naloxone blockade of myocardial ischemic preconditioning does not require central nervous system participation. *Basic Res Cardiol 94*, 36–43.

Chien, S., Oeltgen, P. R., Diana, J. N., Salley, R. K., & Su, T. P. (1994). Extension of tissue survival time in multiorgan block preparation with a delta opioid DADLE ([D-Ala2, D-Leu5]-enkephalin). *J Thorac Cardiovasc Surg 107*, 964–967.

Childers, S. R. (1991). Opioid receptor coupled second messenger systems. *Life Sci 48*, 1991–2003.

Cole, W. C., McPherson, C. D., & Sontag, D. (1991). ATP-regulated K^+ channels protect the myocardium against ischemia/reperfusion damage. *Circ Res 69*, 571–581.

Curtis, M. J., Pugsley, M. K., & Walker, M. J. A. (1993). Endogenous chemical mediators of ventricular arrhythmias in ischaemic heart disease. *Cardiovasc Res 27*, 703–719.

Dana, A., & Yellon, D. M. (1998). Angina: who needs it? Cardioprotection in the preconditioning era. *Cardiovasc Drug Ther 12*, 515–528.

de Bold, A. J. (1985). Atrial natriuretic factor: a hormone produced by the heart. *Science 230*, 767–770.

Dickson, E. W., Blehar, D. J., Tubbs, R. J., Porcaro, W. A., Carraway, R. E., & Przyklenk, K. (2001). Preconditioning induction trigger evokes cardioprotection via the opiate receptor. *Acad Emerg Med 8*, 560–561.

Dohlman, H. G., Thorner, J., Caron, M. G., & Lefkowitz, R. J. (1991). Model systems for the study of seven-transmembrane-segment receptors. *Annu Rev Biochem 60*, 653–688.

duBell, W. H., & Lakatta, E. G. (1991). The effects of the κ-opioid agonist U50,488H on guinea pig ventricular myocytes. *Biophys J 59 (suppl.)*, 465a.

Dumont, M., & Lemaire, S. (1998). Characterization of the high affinity [3H]nociceptin binding site in membrane preparations of rat heart: correlations with the non-opioid dynorphin binding site. *J Mol Cell Cardiol 30*, 2751–2760.

Ela, C., Barg, J., Vogel, Z., Hasin, Y., & Eilam, Y. (1997). Distinct components of morphine effects on cardiac myocytes are mediated by the kappa and delta opioid receptors. *J Mol Cell Cardiol 29*, 711–720.

el Sharkawy, T. Y., al-Shireida, M. F., & Pilcher, C. W. (1991). Vascular effects of some opioid receptor agonists. *Can J Physiol Pharmacol 69*, 846–851.

Evans, C. J., Keith, D. E., Morrison, H., Magendzo, K., & Edwards, R. H.

(1992). Cloning of a delta opioid receptor by functional expression. *Science 258*, 1952–1955.

Fagbemi, O., Lepran, I., Parratt, J. R., & Szekeres, L. (1982). Naloxone inhibits early arrhythmias resulting from acute coronary ligation. *Br J Pharmacol 76*, 504–506.

Fagbemi, O., Kane, K. A., Lepran, I., Parratt, J. R., & Szekeres, L. (1983). Antiarrhythmic actions of meptazinol, a partial agonist at opiate receptors, in acute myocardial ischaemia. *Br J Pharmacol 78*, 455–460.

Feuerstein, G., Powell, E., & Faden, A. I. (1985). Central effects of mu, delta, and kappa receptor agonists in haemorrhagic shock. *Peptides 6*, 11–13.

Fontana, F., Bernardi, P., Pich, E. M., Capelli, M., Bortoluzzi, L., Spampinato, S., & Canossa, M. (1993). Relationship between plasma atrial natriuretic factor and opioid peptide levels in healthy subjects and in patients with acute congestive heart failure. *Eur Heart J 14*, 219–225.

Fowler, C. J., & Fraser, G. L. (1994). μ-, δ-, and κ-opioid receptors and their subtypes. A critical review with emphasis on radioligand binding experiments. *Neurochem Int 24*, 401–426.

Frame, L. H., & Argentieri, T. M. (1985). Naloxone has local anesthetic effects on canine cardiac Purkinje fibres. *Circulation 72*, 234.

Frazier, D. T., Ohta, M., & Narahashi, T. (1973). Nature of the morphine receptor present in the squid axon. *Proc Soc Exp Biol Med 142*, 1209–1214.

Fryer, R. M., Hsu, A. K., Eells, J. T., Nagase, H., & Gross, G. J. (1999). Opioid-induced second window of cardioprotection: potential role of mitochondrial K_{ATP} channels. *Circ Res 84*, 846–851.

Garlid, K. D., Paucek, P., Yarov-Yarovoy, V., Murray, H. N., Darbenzio, R. B., D'Alonzo, A. J., Lodge, N. J., Smith, M. A., & Grover, G. J. (1997). Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels. Possible mechanism of cardioprotection. *Circ Res 81*, 1072–1082.

George, S. R., Fan, T., Xie, Z., Tse, R., Tam, V., Varghese, G., & O'Dowd, B. F. (2000). Oligomerization of mu and delta opioid receptors. *J Biol Chem 275*, 26128–26135.

Goldstein, A., Lowney, L. I., & Pal, B. K. (1971). Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. *Proc Natl Acad Sci USA 68*, 1742–1747.

Greenberg, S., McGowan, C., Xie, J., & Summer, W. R. (1994). Selective pulmonary and venous smooth muscle relaxation by furosemide: a comparison with morphine. *J Pharmacol Exp Ther 270*, 1077–1085.

Gross, G. J., & Auchampach, J. A. (1992). Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ Res 70*, 223–233.

Gross, G. J., & Fryer, R. M. (1999). Sarcolemmal versus mitochondrial ATP-sensitive K^+ channels and myocardial preconditioning. *Circ Res 84*, 973–979.

Grudt, T. J., & Williams, J. T. (1993). κ-Opioid receptors also increase potassium conductance. *Proc Natl Acad Sci USA 90*, 11429–11432.

Guo, Y., Wu, W. J., Qiu, Y., Tang, X. L., Yang, Z., & Bolli, R. (1998). Demonstration of an early and a late phase of ischemic preconditioning in mice. *Am J Physiol 275*, H1375–H1387.

Guo, Y., Jones, W. K., Xuan, Y. T., Tang, X. L., Bao, W., Wu, W. J., Han, H., Laubach, V. E., Ping, P., Yang, Z., Qiu, Y., & Bolli, R. (1999). The late phase of ischemic preconditioning is abrogated by targeted disruption of the inducible NO synthase gene. *Proc Natl Acad Sci USA 96*, 11507–11512.

Hall, E. D., Wolf, D. L., & McCall, R. B. (1988). Cardiovascular depressant effects of the kappa opioid receptor agonists U-50,488H and spiradoline mesylate. *Circ Shock 26*, 409–417.

Harasawa, Y., Kimura, M., & Hayashi, S. (1991). Inhibitory effect of spiradoline, a kappa opioid receptor agonist, on calcium-induced contraction and the intracellular calcium concentration in procine coronary artery. *Cardiovasc Res 25*, 802–806.

Helgesen, K. G., & Refsum, H. (1987). Arrhythmogenic, antiarrhythmic and inotropic properties of opioids. Effects of piritramide, pethidine and morphine compared on heart muscle isolated from rats. *Pharmacology 35*, 121–129.

Henry, S. J., Grandy, D. K., Lester, H. A., Davidson, N., & Chavkin, C. (1995). κ-opioid receptors couple to inwardly rectifying potassium channels when coexpressed by Xenopus oocytes. *Mol Pharmacol 47*, 551–557.

Hicks, M. N., Oldroyd, K. G., & Cobbe, S. M. (1992). Ischaemia, extracellular potassium accumulation and the cardiac electrophysiological effects of *dl* and *d*-naloxone. *Eur Heart J 13 (suppl.)*, SI–SIV.

Himura, Y., Liang, C. S., Imai, N., Delehanty, J. M., Woolf, P. D., & Hood, W. B. (1994). Short-term effects of naloxone on hemodynamics and baroreflex function in conscious dogs with pacing-induced congestive heart failure. *J Am Coll Cardiol 23*, 194–200.

Holaday, J. W. (1983). Cardiovascular effects of endogenous opiate systems. *Annu Rev Pharmacol Toxicol 23*, 541–594.

Holaday, J. W., & Faden, A. I. (1978). Naloxone reversal of endotoxin hypotension suggests a role of endorphins in shock. *Nature 275*, 450–451.

Holtzman, S. G. (1980). Phencyclidine-like discriminative effects of opioids in the rat. *J Pharmacol Exp Ther 214*, 614–619.

Huang, X. D., Lee, A. Y. S., Wong, T. M., Zhan, C. Y., & Zhao, Y. Y. (1986). Naloxone inhibits arrhythmias induced by coronary artery occlusion and reperfusion in anaesthetized dogs. *Br J Pharmacol 87*, 475–477.

Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., & Morris, H. R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature 25*, 577–579.

Hughes, J., Kosterlitz, H. W., & Smith, T. W. (1977). The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues. *Br J Pharmacol 61*, 639–647.

Huidobro-Toro, J. P., & Parada, S. (1985). κ-Opiates and urination: pharmacological evidence for an endogenous role of the κ-opiate receptor in fluid and electrolyte balance. *Eur J Pharmacol 107*, 1–10.

Hung, C. F., Tsai, C. H., & Su, M. J. (1998). Opioid receptor independent effects of morphine on membrane currents in single cardiac myocytes. *Br J Anaesth 81*, 925–931.

Hutchinson, M., Kosterlitz, H. W., Leslie, F. M., & Waterfield, A. A. (1975). Assessment in the guinea pig ileum and mouse vas deferens of benzomorphans which have strong antinociceptive activity but which do not substitute for morphine in the dependent monkey. *Br J Pharmacol 55*, 541–546.

Illes, P., Bettermann, R., Brod, I., & Boucher, B. (1987). Beta-endorphin sensitive opioid receptors in the rat tail artery. *Naunyn Schmeidebergs Arch Pharmacol 335*, 420–427.

Imai, N., Kashiki, M., Woolf, P. D., & Liang, C. S. (1994). Comparison of cardiovascular effects of mu- and delta-opioid receptor antagonists in dogs with congestive heart failure. *Am J Physiol 267*, H912–H917.

Introna, R. P., Bridges, M. T., Yodlowski, E. H., Grover, T. E., & Pruett, J. K. (1995). Direct effects of fentanyl on canine coronary artery rings. *Life Sci 56*, 1265–1273.

Iyengar, S., Kim, H. S., & Wood, P. L. (1985). *Kappa* opiate agonists modulate the hypothalamic–pituitary–adrenocortical axis in the rat. *J Pharmacol Exp Ther 234*, 463–469.

Iyengar, S., Kim, H. S., & Wood, L. (1986). Effects of kappa opiate agonist on neurochemical and neuroendocrine indices: evidence for kappa receptor subtypes. *Life Sci 39*, 637–644.

Jordan, B. A., & Devi, L. A. (1999). G-protein-coupled receptor heterodimerization modulates receptor function. *Nature 399*, 697–700.

Kaschube, M., & Brasch, H. (1991). Negative chronotropic but no antiarrhythmic effect of (+)- and (−)-naloxone in rats and guinea pigs. *Cardiovasc Res 25*, 230–234.

Kasper, E., Ventura, C., Ziman, B. D., Lakatta, E. G., Weisman, H., & Capogrossi, M. C. (1992). Effect of U-50,488H on the contractile response of cardiomypathic hamster ventricular myocytes. *Life Sci 50*, 2029–2035.

Kelly, R. A., Balligand, J. L., & Smith, T. W. (1996). Nitric oxide and cardiac function. *Circ Res 79*, 363–380.

Kieffer, B. L. (1999). Opioids: first lessons from knockout mice. *Trends Pharmacol Sci 20*, 19–26.

Kieffer, B. L., Befort, K., Gaveriaux-Ruff, C., & Hirth, C. G. (1992). The δ-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. *Proc Natl Acad Sci USA 89*, 12048–12052.

Knapp, R. J., Malatynska, E., Fang, L., Li, X., Babin, E., Nguyen, M., Santoro, G., Varga, E. V., Hruby, V. J., Roeske, W. R., & Yamamura, H. (1994). Identification of a human delta opioid receptor: cloning and expression. *Life Sci 54*, PL463–PL469.

Knapp, R. J., Malatynska, E., Collins, N., Fang, L., Wang, J. Y., Hruby, V. J., Roeske, W. R., & Yamamura, H. I. (1995). Molecular biology and pharmacology of cloned opioid receptors. *FASEB J 9*, 516–525.

Komjati, K., Velkei-Harvich, M., Toth, J., Dallos, G., Nyary, I., & Sandor, P. (1996). Endogenous opioid mechanisms in hypothalamic blood flow autoregulation during haemorrhagic hypotension and angiotensin-induced acute hypertension in cats. *Acta Physiol Scand 157*, 53–61.

Kong, H., Raynor, K., Yano, H., Takeda, J., Bell, G. I., & Reisine, T. (1994). Agonists and antagonists bind to different domains of the cloned kappa opioid receptor. *Proc Natl Acad Sci USA 91*, 8042–8046.

Konkoy, C. S., & Childers, S. R. (1989). Dynorphin-selective inhibition of adenylyl cyclase in guinea pig cerebellum membranes. *Mol Pharmacol 36*, 627–633.

Krumins, S. A., Faden, A. E., & Feuerstein, G. (1985). Opiate binding in rat hearts: modulation of binding after hemorrhagic shock. *Biochem Biophys Res Commun 127*, 120–128.

Kunihara, M., Ohyama, M., Nakano, M., & Hayashi, S. (1989). Analgesic activity of spiradoline mesylate (U-62,066E), a kappa opioid agonist in mice. *Life Sci 45*, 1191–1198.

Kunihara, M., Ohyama, M., & Nakano, M. (1993). Effects of spiradoline mesylate, a selective κ-opioid-receptor agonist, on the central dopamine system with relation to mouse locomotor activity and analgesia. *Jpn J Pharmacol 62*, 223–230.

Lahti, R. A., Von Voigtlander, P. F., & Barsuhn, C. (1982). Properties of a selective kappa agonist, U-50,488H. *Life Sci 31*, 2257–2260.

Lai, H. W., Minami, M., Satoh, M., & Wong, Y. H. (1995). G_z coupling to the rat κ-opioid receptor. *FEBS Lett 360*, 97–99.

Lakatta, E. G., Xiao, R., Ventura, C., Guarnieri, C., Spurgeon, H. A., Capogrossi, M. C., & Gambassi, G. (1992). Negative feedback of opioid peptide receptor stimulation on β-adrenergic effects in heart cells. *J Mol Cell Cardiol 24* (*suppl. IV*), S25.

Lang, R. E., Herman, K., Dietz, R., Gaida, W., Ganten, D., Kraft, K., & Unger, Th. (1983). Evidence for the presence of enkephalins in the heart. *Life Sci 32*, 399–406.

Leander, J. D., Zerbe, R. L., & Hart, J. C. (1986). Diuresis and suppression of vasopressin by kappa opioids: comparison with *mu* and *delta* opioids and clonidine. *J Pharmacol Exp Ther 238*, 429–436.

Lee, A. Y. S. (1990). Endogenous opioid peptides and cardiac arrhythmias. *Int J Cardiol 27*, 145–151.

Lee, A. Y. S. (1992). Stereospecific antiarrhythmic effects of naloxone against myocardial ischaemia and reperfusion in the dog. *Br J Pharmacol 107*, 1057–1060.

Lee, A. Y. S., Chen, Y. T., Kan, M. N., P'eng, F. K., Chai, C. Y., & Kuo, J. S. (1992). Consequences of opiate agonist and antagonist in myocardial ischaemia suggest a role of endogenous opioid peptides in ischaemic heart disease. *Cardiovasc Res 24*, 392–395.

Leighton, G. E., Johnson, M. A., Meecham, K. G., Hill, R. G., & Hughes, J. (1987). Pharmacological profile of PD 117,302, a selective κ-opioid agonist. *Br J Pharmacol 92*, 915–922.

Liang, B. T., & Gross, G. J. (1999). Direct preconditioning of cardiac myocytes via opioid receptors and K_{ATP} channels. *Circ Res 84*, 1396–1400.

Liang, C. S., Imai, N., Stone, C. K., Woolf, P. D., Kawashima, S., & Tuttle, R. R. (1987). The role of endogenous opioids in congestive heart failure: effects of nalmefene on systemic and regional hemodynamics in dogs. *Circulation 75*, 443–451.

Light, P. E. (1999). Cardiac KATP channels and ischemic preconditioning: current perspectives. *Can J Cardiol 15*, 1123–1130.

Llobel, F., & Laorden, M. L. (1997). Effects of mu-, delta- and kappa-opioid antagonists in atrial preparations from nonfailing and failing human hearts. *Gen Pharmacol 28*, 371–374.

Lord, J. A. H., Waterfield, A. A., Hughes, J., & Kosterlitz, H. (1977). Endogenous opioid peptides: multiple agonists and receptors. *Nature 267*, 495–499.

Lowe, H. (1991). Role of endogenous opioids in heart failure. *Z Cardiol 80*, 47–51.

Lowenstein, C. J. (1999). NO news is good news. *Proc Natl Acad Sci USA 96*, 10953–10954.

Mackenzie, J. E., Parratt, J. R., & Sitsapesan, R. (1986). The effects of drugs interacting with opioid receptors on ischaemic arrhythmias in anaesthetised rats. *Br J Pharmacol 89*, 614P.

Mansson, E., Bare, L., & Yang, D. (1994). Isolation of a human κ opioid receptor cDNA from placenta. *Biochem Biophys Res Commun 202*, 1431–1437.

Mansour, A., Khachaturian, H., & Lewis, M. E. (1988). Anatomy of CNS opioid receptors. *Trends Neurosci 11*, 308–314.

Mantelli, L., Corti, V., & Ledda, F. (1987). On the presence of opioid receptors in guinea-pig ventricular tissue. *Gen Pharmacol 18*, 309–313.

Martin, N. A., & Prather, P. L. (2001). Interaction of co-expressed mu- and delta-opioid receptors in transfected rat pituitary GH(3) cells. *Mol Pharmacol 59*, 774–783.

Martin, W. R. (1967). Opioid antagonists. *Pharmacol Rev 19*, 464–521.

Martin, W. R. (1984). Pharmacology of opioids. *Pharmacol Rev 35*, 285–323.

Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E., & Gilbert, P. E. (1976). The effects of morphine and nalorphine-like drugs on the non-dependent and morphine-dependent chronic spinal dog. *J Pharmacol Exp Ther 197*, 517–532.

Matteucci, M. D., & Wagner, R. W. (1996). In pursuit of antisense. *Nature 384 (suppl.)*, 20–22.

Matthes, H. W., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., Befort, K., Dierich, A., Le Meur, M., Dolle, P., Tzavara, E., Hanoune, J., Roques, B. P., & Kieffer, B. L. (1996). Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature 383*, 819–823.

Mattia, A., Vanderah, T., Mosberg, I. H., & Porreca, F. (1991). Lack of antinociceptive cross tolerance between [D-Pen^2, D-Pen^5]enkephalin and [D-Ala^2]deltorphin II in mice: evidence for delta receptor subtypes. *J Pharmacol Exp Ther 258*, 583–587.

Mayfield, K. P., & D'Alecy, L. G. (1992). Role of endogenous opioid peptides in the acute adaptation to hypoxia. *Brain Res 582*, 226–231.

Mayfield, K. P., & D'Alecy, L. G. (1994a). Delta-1 opioid receptor dependence of acute hypoxic adaptation. *J Pharmacol Exp Ther 268*, 74–77.

Mayfield, K. P., & D'Alecy, L. G. (1994b). Delta-1 opioid agonist acutely increases hypoxic tolerance. *J Pharmacol Exp Ther 268*, 683–688.

McConnaughey, M. M., Zhai, Q. Z., & Ingenito, A. J. (1998). Effects of rat brain kappa 1- and kappa 2-opioid receptors after chronic treatment with non-peptide kappa-agonists. *J Pharm Pharmacol 50*, 1121–1125.

Meng, F., Xie, G. X., Thompson, R. C., Mansour, A., Goldstein, A., Watson, S. J., & Akil, H. (1993). Cloning and pharmacological characterization of a rat kappa opioid receptor. *Proc Natl Acad Sci USA 90*, 9954–9958.

Meng, F., Hoversten, M. T., Thompson, R. C., Taylor, L., Watson, S. J., & Akil, H. (1995). A chimeric study of the molecular basis of affinity and selectivity of the κ and the δ opioid receptors: potential role of extracellular domains. *J Biol Chem 270*, 12730–12736.

Meunier, J. C., Mollereau, C., Toll, L., Suaudeau, C., Moisand, C., Alvinerie, P., Butour, J. L., Guillemot, J. C., Ferrara, P., Monsarrat, B., Mazarguil, H., Vassart, G., Parmentier, M., & Costentin, J. (1995). Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature 377*, 532–535.

Millington, W. R., Rosenthal, D. W., Unal, C. B., & Nyquist-Battie, C. (1999). Localization of pro-opiomelanocortin mRNA transcripts and peptide immunoreactivity in rat heart. *Cardiovasc Res 43*, 107–116.

Minami, M., Toya, T., Katai, Y., Maekawa, K., Nakamura, S., Onogi, T., Kaneko, S., & Satoh, M. (1993). Cloning and expression of a cDNA for the rat κ-opioid receptor. *FEBS Lett 329*, 291–295.

Moore, S. D., Madma, S. G., Schweitzer, P., & Siggins, R. (1994). Voltage-dependent effects of opioid peptides on hippocampal CA3 pyramidal neurons *in vitro*. *J Neurosci 14*, 809–820.

Murphy, D. B., & Murphy, M. B. (1999). Opioid antagonist modulation of ischaemia-induced ventricular arrhythmias: a peripheral mechanism. *J Cardiovasc Pharmacol 33*, 122–125.

Murry, C. E., Jennings, R. B., & Reimer, K. A. (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation 74*, 1124–1136.

Nishi, M., Takeshima, H., Fukuda, K., Kato, S., & Mori, K. (1993). cDNA cloning and pharmacological characterization of an opioid receptor with high affinities for κ-subtype-selective ligands. *FEBS Lett 330*, 77–80.

Nock, B., Giordano, A. L., Cicero, T. J., & O'Connor, L. H. (1990). Affinity of drugs and peptides for U-69,593-sensitive and U-69,593-insensitive kappa opioid binding sites: the U-69,593-insensitive site appears to be the beta-endorphin-specific epsilon receptor. *J Pharmacol Exp Ther 254*, 412–419.

North, R. A. (1986). Opioid receptor types and membrane ion channels. *Trends Neurosci 11*, 114–117.

North, R. A., Williams, J. T., Surprenant, A., & Christie, M. J. (1987). Mu and delta receptors belong to a family of receptors that are coupled to potassium channels. *Proc Natl Acad Sci USA 84*, 5487–5491.

Nothacker, H. P., Reinscheid, R. K., Mansour, A., Henningsen, R. A., Ardati, A., Monsma, F. J., Jr., Watson, S. J., & Civelli, O. (1996). Primary structure and tissue distribution of the orphanin FQ precursor. *Proc Natl Acad Sci USA 93*, 8677–8682.

Oiso, Y., Iwasaki, Y., Kunikazu, K., Takatsuki, D., & Tomita, A. (1988). Effect of the opioid kappa-receptor agonist U50,488H on the secretion of arginine vasopressin. *Neuroendocrinology 48*, 658–662.

Oldroyd, K. G., Gray, C. E., Carter, R., Harvey, K., Borland, W., Beastall, G., & Cobbe, S. M. (1995). Activation and inhibition of the endogenous opioid system in human heart failure. *Br Heart J 73*, 41–48.

Palmer, L. A., Semenza, G. L., Stoler, M. H., & Johns, R. A. (1998). Hypoxia induces type II NOS gene expression in pulmonary artery endothelial cells via HIF-1. *Am J Physiol 274*, L212–L219.

Parratt, J. R., & Sitsapesan, R. (1986). Stereospecific antiarrhythmic effect of opioid receptor antagonists in myocardial ischaemia. *Br J Pharmacol 87*, 621–627.

Pasternak, G. W. (1993). Pharmacological mechanisms of opioid analgesics. *Clin Neuropharmacology 16*, 1–18.

Pasternak, G. W., & Wood, P. J. (1986). Multiple mu opioid receptors. *Life Sci 38*, 1889–1898.

Penz, W. P., Pugsley, M. K., Hsieh, M. Z., & Walker, M. J. A. (1992). A new ECG measure (RSh) for detecting possible sodium channel blockade in vivo in rats. *J Pharmacol Methods 27*, 51–58.

Periyasamy, S., & Hoss, W. (1990). Kappa opioid receptors stimulate phosphoinositide turnover in rat brain. *Life Sci 47*, 219–225.

Pert, C. B., & Snyder, S. H. (1973). Opiate receptor: demonstration in nervous tissue. *Science 179*, 1011–1014.

Peters, G. R., Ward, N. J., Antal, E. G., Lai, P. Y., & DeMaar, E. W. (1987). Diuretic actions in man of a selective kappa opioid agonist: U-62,066E. *J Pharmacol Exp Ther 240*, 128–131.

Pogozheva, I. D., Lomize, A. L., & Mosberg, H. I. (1998). Opioid receptor three-dimensional structures from distance geometry calculations with hydrogen bonding constraints. *Biophys J 75*, 612–634.

Pokrovsky, V. M., & Osadchiy, O. E. (1995). Regulatory peptides as modulators of vagal influence on cardiac rhythm. *Can J Physiol Pharmacol 73*, 1235–1245.

Portoghese, P. S. (1965). A new concept on the mode of interaction of narcotic analgesics with receptors. *J Med Chem 8*, 609–616.

Pruett, J. K., Blair, J. R., & Adams, R. J. (1991). Cellular and subcellular actions of opioids in the heart. In: F. G. Estafanous (Ed.), *Opioids in Anesthesia*, Vol. II (pp. 61–70). Boston: Butterworth-Heinemann Publishing Corp.

Pugsley, M. K., & Goldin, A. L. (1997). Naloxone blockade of sodium currents expressed in Xenopus oocytes. *Proc West Pharmacol Soc 40*, 65–67.

Pugsley, M. K., & Goldin, A. L. (1999). Molecular analysis of the Na^+ channel blocking actions of the novel class I anti-arrhythmic agent RSD 921. *Br J Pharmacol 127*, 9–18.

Pugsley, M. K., Penz, W. P., Walker, M. J. A., & Wong, T.-M. (1992a). Cardiovascular actions of the kappa receptor agonist, U-50,488H, in the absence and presence of opioid receptor blockade. *Br J Pharmacol 105*, 521–526.

Pugsley, M. K., Penz, W. P., Walker, M. J. A., & Wong, T.-M. (1992b). Antiarrhythmic effects of U-50,488H in rats subject to coronary artery occlusion. *Eur J Pharmacol 212*, 15–19.

Pugsley, M. K., Penz, W. P., & Walker, M. J. A. (1993a). Cardiovascular actions of U-50,488H and related kappa agonists. *Cardiovasc Drug Rev 11*, 151–164.

Pugsley, M. K., Saint, D. A., Penz, W. P., & Walker, M. J. A. (1993b). Electrophysiological and antiarrhythmic actions of the kappa agonist PD129290, and its R,R (+) enantiomer. *PD 129289. Br J Pharmacol 110*, 1579–1585.

Pugsley, M. K., Saint, D. A., & Walker, M. J. A. (1994). An electrophysiological basis for the antiarrhythmic actions of the κ-opioid receptor agonist U-50,488H. *Eur J Pharmacol 261*, 303–309.

Pugsley, M. K., Hayes, E. S., Saint, D. A., & Walker, M. J. A. (1995). Do related kappa agonists produce similar effects on cardiac ion channels? *Proc West Pharmacol Soc 38*, 25–27.

Pugsley, M. K., Saint, D. A., Hayes, E., Kramer, D., & Walker, M. J. A. (1998). The sodium channel blocking properties of spiradoline, a κ-receptor agonist, are responsible for its antiarrhythmic action in the rat. *J Cardiovasc Pharmacol 32*, 863–874.

Pugsley, M. K., Yu, E. J., & Goldin, A. L. (2001). Potent and use-dependent block of cardiac sodium channels by U-50,488H, a benzeneacetamide kappa opioid receptor agonist. *Exp Clin Cardiol 6*, 1–11.

Rashid, S., & Waterfall, J. F. (1979). Effect of antiarrhythmic and analgesic drugs on the effective refractory period of guinea-pig isolated atria and ventricular strips. *J Pharm Pharmacol 31*, 411–412.

Raynor, K., Kong, H., Chen, Y., Yasuda, K., Yu, L., Bell, G. I., & Reisine, T. (1994). Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. *Mol Pharmacol 45*, 330–334.

Reinscheid, R. K., Nothacker, H. P., Bourson, A., Ardati, A., Henningsen, R. A., Bunzow, J. R., Grandy, D. K., Langen, H., Monsma, F. J., Jr., & Civelli, O. (1995). Orphanin FQ: a neuropeptide that activates an opioid-like G protein-coupled receptor. *Science 270*, 792–794.

Reisine, T., & Bell, G. I. (1993). Molecular biology of opioid receptors. *Trends Neurosci 16*, 506–510.

Rimoy, G. H., Bhaskar, N. K., Wright, D. M., & Rubin, P. C. (1991). Mechanism of diruetic action of spiradoline (U-62,066E)—a kappa opioid receptor agonist in the human. *Br J Pharmacol 32*, 611–615.

Rubino, A., & Yellon, D. M. (2000). Ischemic preconditioning of the vasculature: an overlooked phenomenon for protecting the heart? *Trends Pharmacol Sci 21*, 225–230.

Sagy, M., Shavit, G., Oron, Y., Vedne, B. A., Gitter, S., & Sarne, Y. (1987). Nonopiate effect of naloxone on cardiac muscle contractility. *J Cardiovasc Pharmacol 9*, 682–685.

Sakamoto, S., Stone, C. K., Woolf, P. D., & Liang, C. S. (1989). Opiate receptor antagonism in right-sided congestive heart failure. Naloxone exerts salutary hemodynamic effects through its action on the central nervous system. *Circ Res 65*, 103–114.

Sarne, Y., Flitstein, A., & Oppenheimer, E. (1991). Anti-arrhythmic activities of opioid agonists and antagonists and their stereoisomers. *Br J Pharmacol 102*, 696–698.

Schultz, J. E., Rose, E., Yao, Z., & Gross, G. J. (1995). Evidence for involvement of opioid receptors in ischemic preconditioning in rat hearts. *Am J Physiol 268*, H2157–H2161.

Schultz, J. J., Hsu, A. K., & Gross, G. J. (1996). Morphine mimics the

cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart. *Circ Res 78*, 1100–1104.

Schultz, J. J., Hsu, A. K., & Gross, G. J. (1997a). Ischemic preconditioning is mediated by a peripheral opioid receptor mechanism in the intact rat heart. *J Mol Cell Cardiol 29*, 1355–1362.

Schultz, J. J., Hsu, A. K., & Gross, G. J. (1997b). Ischemic preconditioning and morphine-induced cardioprotection involve the delta (δ)-opioid receptor in the intact rat heart. *J Mol Cell Cardiol 29*, 2187–2195.

Schultz, J. E., Hsu, A. K., & Gross, G. J. (1998a). Ischemic preconditioning in the intact rat heart is mediated by delta1- but not mu- or kappa-opioid receptors. *Circulation 97*, 1282–1289.

Schultz, J. el-J., Hsu, A. K., Nagase, H., & Gross, G. J. (1998b). TAN-67, a δ_1-opioid receptor agonist, reduces infarct size via activation of $G_{i/o}$ proteins and K_{ATP} channels. *Am J Physiol 274*, H909–H914.

Shen, S., & Ingenito, A. J. (1999a). Comparison of cardiovascular responses to intra-hippocampal mu, delta and kappa opioid agonists in spontaneously hypertensive rats and isolation-induced hypertensive rats. *J Hypertens 17*, 497–505.

Shen, S., & Ingenito, A. J. (1999b). Depressor effect of kappa opioid agonist on hypertension induced by isolation in the rat. *Clin Exp Hypertens 21*, 275–297.

Simonin, F., Valverde, O., Smadja, C., Slowe, S., Kitchen, I., Dierich, A., Le Meur, M., Roques, B. P., Maldonado, R., & Kieffer, B. L. (1998). Disruption of the kappa-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective kappa-agonist U-50,488H and attenuates morphine withdrawal. *EMBO J 17*, 886–897.

Sitsapesan, R., & Parratt, J. R. (1989). The effects of drugs interacting with opioid receptors in the early ventricular arrhythmias arising from myocardial ischaemia. *Br J Pharmacol 97*, 795–800.

Smart, D., & Lambert, D. G. (1996). The stimulatory effects of opioids and their possible role in the development of tolerance. *Trends Pharmacol Sci 17*, 264–269.

Sora, I., Takahashi, N., Funada, M., Ujike, H., Revay, R. S., Donovan, D. M., Miner, L. L., & Uhl, G. R. (1997). Opiate receptor knockout mice define mu receptor roles in endogenous nociceptive responses and morphine-induced analgesia. *Proc Natl Acad Sci USA 94*, 1544–1549.

Sun, S. Y., Liu, Z., Li, P., & Ingenito, A. J. (1996). Central effects of opioid agonists and naloxone on blood pressure and heart rate in normotensive and hypertensive rats. *Gen Pharmacol 27*, 1187–1194.

Szmuszkovicz, J., & Von Voigtlander, P. (1982). Benzeneacetamide amines: structurally novel non-μ opioids. *J Med Chem 25*, 1125–1126.

Tai, K. K., Jin, W.-Q., Chan, T. K. Y., & Wong, T. M. (1991). Characterization of [^{3}H]U69,593 binding sites in the rat heart by receptor binding assays. *J Mol Cell Cardiol 23*, 1297–1302.

Takano, H., Manchikalapudi, S., Tang, X. L., Qiu, Y., Rizvi, A., Jadoon, A. K., Zhang, Q., & Bolli, R. (1998). Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits. *Circulation 98*, 441–449.

Timmis, A. D., Rothman, M. T., Henderson, M. A., Geal, P. W., & Chamberlain, D. A. (1980). Haemodynamic effects of intravenous morphine in patients with acute myocardial infarction complicated by severe left ventricular failure. *Br Med J 280*, 980–982.

Tsuchida, A., Miura, T., Tanno, M., Nozawa, Y., Kita, H., & Shimamoto, K. (1998). Time window for the contribution of the delta-opioid receptor to cardioprotection by ischemic preconditioning in the rat heart. *Cardiovasc Drugs Ther 12*, 365–373.

Uchida, S., Suzuki, A., Hotta, H., & Sato, A. (1999). Cardiac actions of opioids. *Neurosci Lett 269*, 161–164.

Ulens, C., Daenens, P., & Tytgat, J. (1999). Norpropoxyphene-induced cardiotoxicity is associated with changes in ion-selectivity and gating of HERG currents. *Cardiovasc Res 44*, 568–578.

Utz, J., Eckert, R., & Trautwein, W. (1995). Inhibition of L-type calcium currents in guinea pig ventricular myocytes by the kappa-opioid agonist U50488H does not involve binding to opiate receptors. *J Pharmacol Exp Ther 274*, 627–633.

Vargish, T., & Beamer, K. C. (1989). Delta and mu receptor agonists correlate with greater depression of cardiac function than morphine sulfate in perfused rat hearts. *Circ Shock 27*, 245–251.

Ventura, C., Bastagli, L., Bernardi, P., Caldarera, C. M., & Guarnieri, C. (1989). Opioid receptors in rat cardiac sarcolemma: effect of phenylephrine and isoproterenol. *Biochim Biophys Acta 987*, 69–74.

Von Voigtlander, P. F., & Lewis, R. A. (1982a). A comparison of putative kappa receptor agonists: analgesic mechanisms and narcotic antagonist activity in mice. *Fed Proc 41*, 1314.

Von Voigtlander, P. F., & Lewis, R. A. (1982b). U-50,488H, a selective kappa opioid agonist: comparison to other reputed kappa agonists. *Prog Neuropsychopharmacol Biol Psychiatry 6*, 467–470.

Von Voigtlander, P. F., Collins, R. J., Lewis, R. A., & Neff, G. L. (1981). U-50,488H (trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide): prototype for a new class of opioid analgesics. *Pharmacologist 23*, 134.

Von Voigtlander, P. F., Lahti, R. A., & Ludens, J. H. (1982). U-50,488H: a selective and structurally novel non-*mu* (*fs*) opioid agonist. *J Pharmacol Exp Ther 224*, 7–12.

Wang, J. B., Imai, Y., Eppler, C. M., Gregor, P., Spivak, C. E., & Uhl, G. R. (1993). Mu opiate receptor: cDNA cloning and expression. *Proc Natl Acad Sci USA 90*, 10230–10234.

Wang, J. Q., & Ingenito, A. J. (1994). Receptors mediating depressor responses to intrahippocampal injection of L-glutamate and dynorphin-A(1–8) in conscious, spontaneously hypertensive and normotensive Wistar–Kyoto rats. *J Cardiovasc Pharmacol 24*, 999–1003.

Watson, S. J., Akil, H., Fischli, W., Goldstein, A., Zimmerman, E., Nilaver, G., & van wimersma Griedanus, T. B. (1982). Dynorphin and vasopressin: common localization in magnocellular neurons. *Science 216*, 85–87.

Wegener, K., & Kummer, W. (1994). Sympathetic noradrenergic fibers as the source of immunoreactive alpha-neoendorphin and dynorphin in the guinea pig heart. *Acta Anat (Basel) 151*, 112–119.

Weihe, E., McKnight, A. T., Corbett, A. D., & Kosterlitz, H. W. (1985). Proenkephalin- and prodynorphin-derived opioid peptides in guinea-pig heart. *Neuropeptides 5*, 453–456.

Wittert, G., Hope, P., & Pyle, D. (1996). Tissue distribution of opioid receptor gene expression in the rat. *Biochem Biophys Res Commun 218*, 877–881.

Wong, T. M., & Lee, Y. S. (1985). Cardiac antiarrhythmic evolution of naloxone with or without propranolol using a modified chloroform-hypoxia screening test in the rat. *Clin Exp Pharmacol Physiol 12*, 379–385.

Wong, T. M., Lee, Y. S., & Tai, K. K. (1990). Effects of drugs interacting with opioid receptors during normal perfusion or ischemia and reperfusion in the isolated rat heart—an attempt to identify cardiac opioid receptor subtype(s) involved in arrhythmogenesis. *J Mol Cell Cardiol 22*, 1167–1175.

Wright, R. C., McConnaughey, M. M., Phan, T. A., & Ingenito, A. J. (1999). Kappa-opioid receptor antisense oligonucleotide injected into rat hippocampus causes hypertension. *Eur J Pharmacol 377*, 57–61.

Wu, C., Fry, C. H., & Henry, J. (1997). The mode of action of several opioids on cardiac muscle. *Exp Physiol 82*, 261–272.

Xia, Q., Sheng, J. Z., Tai, K. K., & Wong, T. M. (1994). Effects of chronic U50,488H treatment on binding and mechanical responses of the rat hearts. *J Pharmacol Exp Ther 268*, 930–934.

Xie, G., Meng, F., Mansour, A., Thompson, R. C., Hoversten, M. T., Golkstein, A., Watson, S. J., & Akil, H. (1994). Primary structure and functional expression of a guinea pig κ opioid (dynorphin) receptor. *Proc Natl Acad Sci USA 91*, 3779–3783.

Xu, H., Partilla, J. S., de Costa, B. R., Rice, K. C., & Rothman, R. B. (1993). Differential binding of opioid peptides and other drugs to two subtypes of opioid delta ncx binding sites in mouse brain: further evidence for delta receptor heterogeneity. *Peptides 14*, 893–907.

Xuan, Y. T., Tang, X. L., Banerjee, S., Takano, H., Li, R. C., Han, H., Qiu, Y., Li, J. J., & Bolli, R. (1999). Nuclear factor-kappa B plays an essential

role in the late phase of ischemic preconditioning in conscious rabbits. *Circ Res 84*, 1095–1109.

Xue, J. C., Chen, C., Zhu, J., Kunapuli, S., DeRiel, J. K., Yu, L., & Liu-Chen, L. Y. (1994). Differential binding domains of peptide and non-peptide ligands in the cloned rat kappa opioid receptor. *J Biol Chem 269*, 30195–30199.

Yamada, K., Imai, M., & Yoshida, S. (1989). Mechanism of diuretic action of U-62,066E, a κ opioid receptor agonist. *Eur J Pharmacol 160*, 229–237.

Yamada, K., Nakano, M., & Yoshida, S. (1990). Inhibition of elevated arginine vasopressin secretion in response to osmotic stimulation and acute haemorrhage by U-62,066E, a κ-opioid receptor agonist. *Br J Pharmacol 99*, 384–388.

Yasuda, K., Raynor, K., Kong, H., Breder, C. D., Takeda, J., Reisine, T., & Bell, G. I. (1993). Cloning and functional comparison of kappa and delta opioid receptors from mouse brain. *Proc Natl Acad Sci USA 90*, 6736–6740.

Yong, S. L., Abraham, S., Pugsley, M. K., Hayes, E. S., Zolotoy, A. B., & Walker, M. J. A. (1996). SAR evidence that antiarrhythmic activity is unrelated to opioid kappa agonist activity. *Br J Pharmacol 119*, P35.

Zhan, Z. Y., Lee, A. Y. S., & Wong, T. M. (1985). Naloxone blocks the cardiac effects of myocardial ischemia and reperfusion in the rat isolated heart. *Clin Exp Pharmacol Physiol 12*, 373–378.

Zhang, W. M., Jin, W. Q., & Wong, T. M. (1996). Multiplicity of kappa opioid receptor binding in the rat cardiac sarcolemma. *J Mol Cell Cardiol 28*, 1547–1554.

Zhu, Y., Hsu, M. S., & Pintar, J. E. (1998). Developmental expression of the mu, kappa, and delta opioid receptor mRNAs in mouse. *J Neurosci 18*, 2538–2549.