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Influence of the optical isomers (+)- and (−)-naloxone on beating frequency, contractile force and action potentials of guinea-pig isolated cardiac preparations

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1 Naloxone in concentrations ranging from 7.5 to 120 μmol l^{-1} reduced the beating frequency of guinea-pig isolated atria. The ED_{50} was 7.9 μmol l^{-1} for the (−)-isomer and 10.8 μmol l^{-1} for the (+)-isomer.

2 Concentrations up to 120 μmol l^{-1} of either (−)- or (+)-naloxone did not affect the force of contraction of left atria stimulated at 1 Hz.

3 In concentrations from 30 to 120 μmol l^{-1} (−)-naloxone increased the action potential (AP) duration and the functional refractory period (FRP) of papillary muscles. The resting membrane potential and the AP amplitude remained unchanged, while a small decrease of $\dot{V}_{max}$ was seen with the larger drug concentrations. The influence of (+)-naloxone (120 μmol l^{-1}) was comparable to that of the (−)-isomer.

4 The influence of morphine (120 μmol l^{-1}) on papillary muscle AP was small. AP duration and FRP showed a marginal prolongation while $\dot{V}_{max}$ was slightly decreased.

5 (−)-Naloxone 60 μmol l^{-1} had no effect on slow-response APs of K^+-depolarized papillary muscles. Slow-response APs were abolished by verapamil (1 μmol l^{-1}).

6 In left atrial strips the prolongation of the AP duration produced by 120 μmol l^{-1} of either (−)- or (+)-naloxone resembled the drug effect in papillary muscles.

7 Most of the observed changes can be explained by an inhibition of the time-dependent membrane K^+ outward current, an effect of naloxone that may be classified as a Class III antiarrhythmic action. Apparently this effect is not mediated by stereospecific opioid receptors.

Introduction

Injections of the opioid antagonist naloxone have occasionally produced arrhythmias in man (Pallasch & Gill, 1981). In anaesthetized rats, naloxone enhanced noradrenaline-induced arrhythmias (Rabkin & Fung, 1984) but prevented disorders of rhythm resulting from coronary artery ligation (Fagbemi *et al.*, 1982). In pigs, on the other hand, the drug was ineffective against occlusion-induced arrhythmias (Bergey & Beil, 1983). More than one explanation can be offered for the influence of naloxone on the cardiac rhythm. First, the drug seems to block opioid receptors in the central nervous system and in this way increases the activity of sympathetic nerves and the outflow of catecholamines from the adrenal gland (Holaday *et al.*, 1983). The resulting sympathetic stimulation of the heart seems to be beneficial in various models of experimental shock (see Holaday, 1983, for review) but may also cause dysrhythmias. Second, the heart itself possesses opioid receptors (Saxon *et al.*, 1982) and stores opioid peptides (Lang *et al.*, 1983; Weihe *et al.*, 1983). Gautret & Schmitt (1984) reported that κ-opioid receptors are involved in the regulation of heart rate and blood pressure of rats. In field-stimulated guinea-pig atria, presynaptic opioid receptors seem to modulate stimulation-evoked noradrenaline release (Ledda & Mantelli, 1982; Ledda *et al.*, 1984). In addition, postsynaptic opioid receptors regulate the sensitivity of isolated atria towards exogenously administered noradrenaline (Eiden & Ruth, 1982; Ruth & Eiden, 1984; Ruth *et al.*, 1984). Thus, by blocking cardiac opioid receptors, naloxone could also influence heart rate or disorders of rhythm. Third, there are some hints that large concentrations of naloxone may have a direct negative chronotropic

effect on the myocardium which does not involve specific receptors (Montel & Starke, 1973; Feria *et al.*, 1982). The present experiments with the optical isomers (+)- and (−)-naloxone were undertaken to differentiate between direct and receptor-mediated effects of the drug in guinea-pig isolated cardiac preparations.

Methods

Experimental procedure

Male guinea-pigs (250–400 g) were killed by a blow on the head and the hearts rapidly removed. Right or left atria, papillary muscles from the right ventricle or left atrial strips (approximately 0.2 × 0.5 cm) were dissected in cooled oxygenated Krebs-Henseleit solution of the following composition (mmol l^{-1}: NaCl 117.6, KCl 5.8, $CaCl_2$ 2.5, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgSO_4$1.2 and glucose 5.5.

Right or left atria were mounted in a 10 ml organ bath filled with Krebs-Henseleit solution kept at 32° ± 0.2°C and constantly gassed with 95% O_2 and 5% CO_2. The resting tension was adjusted to 9.81 mN. Right atria were allowed to beat spontaneously while left atria were paced with square pulses of 1 ms duration at a rate of 1 Hz via a pair of pointed electrodes; stimuli were 1.5 times threshold voltage. The force of contraction was registered isometrically by a K 30 force-displacement transducer (Hugo Sachs, Hugstetten, FRG) on a Hellige Helcoscriptor HE 16 (Hellige, Freiburg, FRG). After an equilibration period of 1 h drugs were added cumulatively in 15 min intervals. A washout period of 15–30 min followed after the highest drug concentration.

For the registration of action potentials (AP), papillary muscles or atrial strips were fixed horizontally in a tissue bath of 20 ml capacity filled with Krebs-Henseleit solution at 32° ± 0.2°C and were stimulated at a rate of 1 Hz as described above. The APs were recorded with standard glass microelectrodes filled with 3 mol l^{-1} KCl and having a tip resistance of 5–10 mΩ. The AP and the maximum depolarization velocity ($\dot{V}_{max}$) were displayed on a Tektronix 502 A dual-beam oscilloscope (Tektronix Inc. Beaverton, OR; USA). To determine the functional refractory period and the reactivation kinetics of $\dot{V}_{max}$ in papillary muscles, an extra stimulus of 3 times threshold voltage was inserted with a variable time interval after every sixth regular stimulus. A detailed description of the registration and evaluation procedures, including the calculation of the half-life of recovery of $\dot{V}_{max}$, has been given in previous papers (Iven & Brasch, 1977; Brasch, 1983).

To induce Ca^{2+}-mediated slow-response APs in papillary muscles, the K^+ concentration of the Krebs-Henseleit solution was raised to 22 mmol l^{-1} and orciprenaline (1 μmol l^{-1}) was added to the organ bath. The muscles were then stimulated at 0.1 Hz with pulses of 2 ms duration and slightly above the threshold voltage (5–7 V).

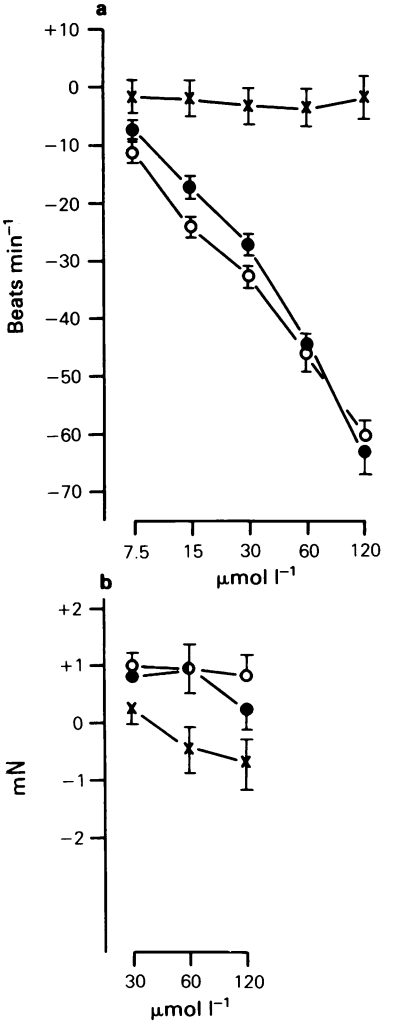

Figure 1 Influence of naloxone on spontaneous rate of beating (right atria) and force of contraction (left atria, stimulation frequency 1 Hz) of guinea-pig atria. The symbols indicate the mean change of the beating frequency (a) or of the contractile force (b) and the corresponding standard error (mean ± s.e.mean; $n = 6$). Abscissa scale: drug concentration (μmol l^{-1}). Ordinate scale: change of beating frequency (beats min^{-1}; a) or change of contractile force (mN; b). (×) Control experiments, no drug; (○) (−)-naloxone; (●) (+)-naloxone.

An equilibration period of 90 min was allowed before drugs were added to the organ bath. Drug effects were observed for 30 min. The functional refractory period and the recovery kinetics of $\dot{V}_{max}$ were determined immediately before and 30 min after drug application. In some experiments the period of drug exposure was followed by a washout period of 20 min with drug-free Krebs-Henseleit solution. Each muscle received only one concentration of one drug. Only those experiments were evaluated in which microelectrode recordings could be obtained from the same cell during the whole time course of the experiment.

Drugs

The following drugs were used: orciprenaline sulphate (Alupent, Boehringer, Ingelheim, FRG); verapamil hydrochloride (Isoptin, Knoll, Ludwigshafen, FRG); morphine hydrochloride (commercial); (−)-naloxone hydrochloride (Du Pont de Nemours, Wilmington, Delaware, USA) and (+)-naloxone hydrochloride (National Institute on Drug Abuse, Rockville, USA).

Statistics

For each parameter measured in the different experimental conditions the pre-drug values in the different treatment groups were compared by analysis of variance (Bartlett's and Scheffe's tests). No significant differences were found. For each single experiment the drug effects on the different parameters were expressed as the changes from the corresponding individual pre-drug control values. Student's t test for paired data was then used to assess the statistical significance of the observed mean changes in each group of six experiments with identical drug treatment. $P < 0.05$ for the two-tailed test was fixed as the criterion for acceptance of statistical significance throughout. For the negative chronotropic effect of naloxone in atria an ED_{50} (i.e. the dose that would produce 50% of the maximal observed reduction of the beating frequency) was calculated from a log concentration-effect curve after linearization by probit transformation and estimation of the regression function with the least squares method. Regression lines were compared as described by Documenta Geigy (1968).

Results

Chronotropic and inotropic effects in atria

The beating frequency of right atria was 140.7 ± 5.4 (mean ± s.e.mean; $n = 6$) beats per minute (beats min^{-1}) at the beginning of control experiments and

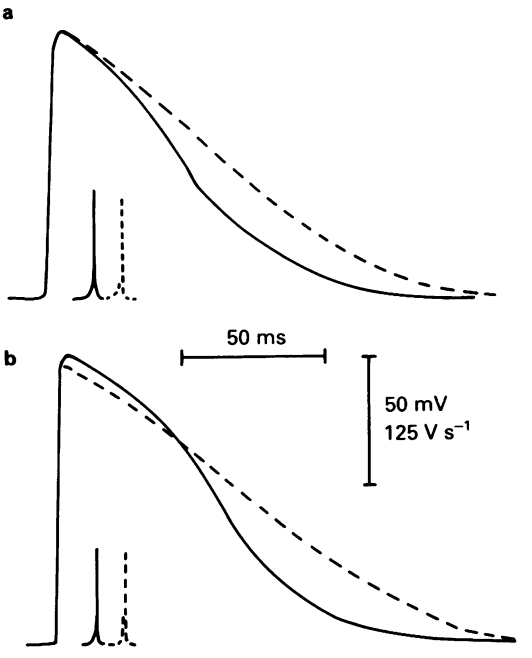

Figure 2 Influence of (−)-naloxone 120 μmol l^{-1} (a) and (+)-naloxone 120 μmol l^{-1} (b) on AP and V_{max} of guinea-pig left atrial strips. Solid lines: AP and $\dot{V}_{max}$ before drug application. Broken lines: AP and V_{max} from the same cell 30 min after application of naloxone.

was not significantly changed by solvent application (Figure 1). In six experiments naloxone had a negative chronotropic effect that became apparent with a drug concentration of 7.5 μmol l^{-1} and was maximal at 120 μmol l^{-1} (Figure 1). The highest concentration of (−)-naloxone reduced the beating frequency from 146.7 ± 3.4 to 86.0 ± 3.5 beats min^{-1} while (+)-naloxone caused a decrease from 139.0 ± 4.3 to 75.7 ± 1.9 beats min^{-1}. From the concentration-response curves (Figure 1) an ED_{50} (the concentration expected to produce 50% of the observed maximal frequency reduction) of 7.9 μmol l^{-1} was calculated for the (−)-isomer and 10.8 μmol l^{-1} for the (+)-isomer. Though small, this difference was statistically significant.

The inotropic effect of 30, 60 and 12 μmol l^{-1} naloxone was tested in 6 left atrial preparations (Figure 1). Although in control experiments the force of contraction declined from 9.53 ± 0.75 to 8.78 ± 0.53 mN, the highest concentration of (−)-naloxone appeared to cause a small increase from 10.04 ± 0.65 to 10.95 ± 1.09 mN and a similar augmentation (from 9.55 ± 0.70 to 9.80 ± 0.72 mN) was observed with 120 μmol l^{-1} (+)-naloxone. However, none of these changes was statistically significant.

Effects on the action potential of atrial strips

Naloxone (120 μmol l^{-1}) increased the AP duration of left atrial strips; 6 experiments were performed with each isomer. The effect developed with a half time of 5.0 min and was maximal 30 min after drug application; (−)- and (+)-naloxone were almost equipotent. Figure 2 presents the outcome of two typical experiments and Table 1 summarizes the results. The prolongation of the AP duration was minimal at the plateau level (measured at 20% of full repolarization) but, at 80% of full repolarization, the AP duration increased from 64.1 ± 3.7 ms to 91.5 ± 5.9 ms with (−)-naloxone and from 66.0 ± 1.8 ms to 97.2 ± 3.0 ms with (+)-naloxone. The membrane resting potential and the AP amplitude remained unchanged and the small (5%) decrease of $\dot{V}_{max}$ was statistically significant only with the (−)-isomer.

Effects on the action potential of papillary muscles

In papillary muscles six experiments were performed at each concentration of the two drugs. (−)-Naloxone (30, 60 and 120 μmol l^{-1}) increased the AP duration but (+)-naloxone (120 μmol l^{-1}) seemed to be even more effective in this respect (Table 2). As in atria, the AP prolongation was most prominent at 80% of full repolarization (Figure 3). With (−)-naloxone 120 μmol l^{-1}, the AP duration was increased from 206.7 ± 9.4 ms to 234.2 ± 11.0 ms, whereas the same concentration of (+)-naloxone caused an increase from 199.2 ± 8.1 ms to 242.5 ± 15.9 ms. The effect developed with a half time of 4.1 ± 5.1 min, was maximal 30 min after drug application and was readily reversible after washout (Table 2). Naloxone did not affect the membrane resting potential or the AP amplitude but a small reduction of $\dot{V}_{max}$ was observed with the highest concentration of either isomer. The increase of the AP duration was accompanied by a similar lengthening of the functional refractory period (Table 2). $\dot{V}_{max}$ for the first AP after the end of the functional refractory period was smaller than $\dot{V}_{max}$ for the usual APs, indicating that the fast Na^+ channel

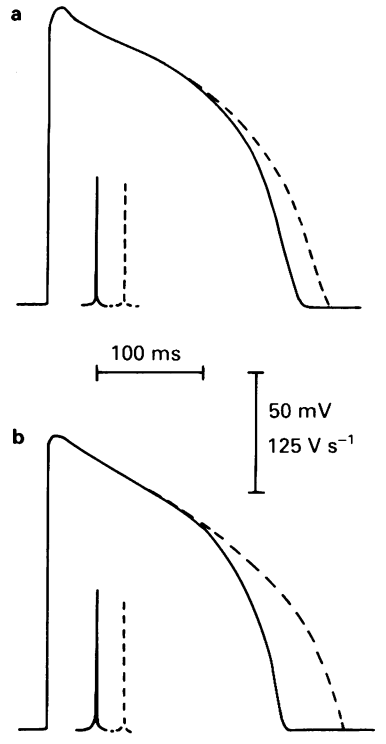

Figure 3 Influence of (−)-naloxone 120 μmol l^{-1} (a) and (+)-naloxone 120 μmol l^{-1} (b) on AP and $\dot{V}_{max}$ of guinea-pig papillary muscles. Solid lines: AP and $\dot{V}_{max}$ before drug application. Broken lines: AP and $\dot{V}_{max}$ from the same cell 30 min after application of naloxone.

Table 1 Influence of naloxone on action potential parameters of guinea-pig left atrial strips

Treatment		*RMP* (mV)	*APA* (mV)	$\dot{V}_{max}$ (V s^{-1})	APD_{20} (ms)	APD_{80} (ms)
None	Control	−77.8 ± 0.6	102.4 ± 1.5	116.6 ± 9.2	19.6 ± 2.5	63.8 ± 4.0
	Δ 30 min	−0.2 ± 0.5	−0.2 ± 0.7	−1.3 ± 1.8	+0.7 ± 0.3	0.0 ± 1.2
(−)-Naloxone 120 μmol l^{-1}	Control	−78.8 ± 0.5	101.2 ± 0.9	105.6 ± 7.4	18.7 ± 1.7	64.1 ± 3.7
	Δ 30 min	−1.0 ± 0.7	−0.2 ± 0.3	−5.3 ± 0.8*	+5.0 ± 1.2*	+27.3 ± 2.7*
(+)-Naloxone 120 μmol l^{-1}	Control	−77.7 ± 0.6	103.7 ± 1.1	140.8 ± 20.0	21.0 ± 2.1	66.0 ± 1.8
	Δ 30 min	−0.8 ± 1.1	−1.1 ± 0.7	−6.4 ± 4.2	+2.5 ± 1.2	+31.2 ± 2.3*

Pre-drug values (Control) and their changes 30 min after exposure to the drug (Δ 30 min) are given (mean ± s.e.mean; $n = 6$). Asterisks indicate changes that are statistically significant ($P < 0.05$). Abbreviations: RMP = resting membrane potential; APA = action potential amplitude; $\dot{V}_{max}$ = maximum depolarization velocity; APD_{20}, APD_{80} = action potential duration at 20% and 80% of full repolarization.

Table 2 Influence of naloxone and morphine on action potential parameters of guinea-pig papillary muscles

Treatment		*RMP* (mV)	*APA* (mV)	$\dot{V}_{max}$ (V s^{-1})	APD_{20} (ms)	APD_{80} (ms)	*FRP* (ms)
None	Control	−81.7 ± 0.7	116.6 ± 0.7	97.1 ± 6.9	122.5 ± 4.9	211.7 ± 7.8	236.7 ± 11.8
	Δ30 min	+0.7 ± 0.5	0.0 ± 0.2	+1.1 ± 1.1	0.0 ± 0.0	+1.7 ± 2.1	+3.3 ± 2.1
(−)-Naloxone	Control	−79.3 ± 0.3	116.6 ± 0.9	110.2 ± 8.4	109.7 ± 8.0	197.3 ± 11.9	216.7 ± 13.2
30 μmol l^{-1}	Δ30 min	+1.0 ± 0.4	−0.2 ± 0.4	+0.2 ± 2.1	+2.3 ± 2.9	+8.7 ± 3.0*	+8.3 ± 2.1*
(−)-Naloxone	Control	−80.5 ± 1.0	117.7 ± 0.9	124.0 ± 9.5	120.5 ± 10.2	203.8 ± 12.2	220.8 ± 13.7
60 μmol l^{-1}	Δ30 min	+0.3 ± 0.5	0.0 ± 0.2	−4.6 ± 0.7*	−0.7 ± 2.1	+18.5 ± 3.2*	+18.3 ± 3.3*
(−)-Naloxone	Control	−81.8 ± 0.9	119.9 ± 2.0	105.4 ± 9.2	130.0 ± 8.6	206.7 ± 9.4	215.0 ± 8.9
120 μmol l^{-1}	Δ30 min	+0.3 ± 0.5	−2.0 ± 0.4*	−6.4 ± 1.6*	+4.2 ± 2.4	+27.5 ± 2.8*	+33.3 ± 2.8*
	ΔW	0.0 ± 1.0	−0.4 ± 0.6	+1.1 ± 4.4	−4.0 ± 1.0*	+6.0 ± 2.9	+11.0 ± 1.9*
(−)-Naloxone	Control	−84.2 ± 0.6	119.9 ± 0.4	109.1 ± 14.5	130.8 ± 5.1	199.2 ± 8.1	209.2 ± 11.1
120 μmol l^{-1}	Δ30 min	−0.5 ± 0.7	−1.1 ± 0.7	−11.9 ± 4.6*	+12.5 + 4.0*	+43.3 ± 8.2*	+57.5 ± 8.4*
	ΔW	0.0 ± 1.9	+1.1 ± 0.4	−3.3 ± 3.7	−10.0 ± 3.5*	+8.0 ± 4.9	+13.0 ± 6.4
Morphine	Control	−82.2 ± 0.6	115.9 ± 0.6	108.3 ± 6.4	115.8 ± 11.0	213.3 ± 7.9	228.3 ± 8.1
120 μmol l^{-1}	Δ30 min	−0.2 ± 0.5	−1.4 ± 0.3*	−6.7 ± 1.7*	+5.8 ± 3.0	+8.3 ± 1.0*	+5.0 ± 1.8*

Pre-drug values (Control) and their changes 30 min after exposure to the drug (Δ30 min) and after the following washout period (ΔW) are given (mean ± s.e.mean; $n = 6$). Asterisks indicate changes that are statistically significant ($P < 0.05$). FRP = functional refractory period. The other abbreviations are the same as in Table 1.

had not yet been fully reactivated. The subsequent recovery from inactivation was tested with twin pulses (see Methods) in each cell before and 30 min after drug application. The half-life of recovery from inactivation was 14.9 ± 1.9 ms under control conditions and was not significantly changed by 120 μmol l^{-1} of either (−)- or (+)-naloxone.

In an additional series of six experiments, the influence of the μ-opioid receptor agonist, morphine (120 μmol^{-1}), was tested on papillary muscle AP. The observed changes were comparable to the effect of naloxone, but the AP lengthening was less prominent. At 80% of full repolarization the AP duration increased from 213 ± 7.9 ms to 221.7 ± 8.0 ms. This was accompanied by a similar increase of the functional refractory period while $\dot{V}_{max}$ decreased by nearly 6% (Table 2).

Effects on slow-response action potential of papillary muscles

In K^+-depolarized muscles the upstroke of the AP is mediated by the slow inward Ca^{2+} current (I_{si}). In six experiments (−)-naloxone (60 μmol l^{-1}) did not reduce $\dot{V}_{max}$ or the amplitude of slow-response APs (Table 3). The calcium channel blocker, verapamil (1 μmol l^{-1}), on the other hand, quickly reduced $\dot{V}_{max}$ and AP amplitude (Table 3) and rendered the preparation inexcitable 10 to 15 min after drug application. Thus, naloxone apparently did not block the slow inward Ca^{2+} current.

Table 3 Influence of naloxone and varapamil on slow response action potentials of K^+-depolarized papillary muscles

Treatment		*RMP* (mV)	*APA* (mV)	$\dot{V}_{max}$ (V s^{-1})	APD_{80} (ms)
(−)-Naloxone	Control	−50.8 ± 1.5	80.7 ± 0.9	8.0 ± 0.6	280.0 ± 19.0
60 μmol l^{-1}	Δ 30 min	+1.0 ± 0.5	+0.5 ± 1.0	−1.2 ± 0.6	+3.3 ± 13.4
Verapamil	Control	−54.0 ± 1.2	79.3 ± 2.5	8.5 ± 1.3	280.8 ± 16.2
1 μmol l^{-1}	Δ 10 min	+0.5 ± 0.3	−6.8 ± 1.7*	−3.0 ± 1.0*	−39.0 ± 9.0*

Pre-drug values (Control) and their changes after a 30 min (with naloxone) or 10 min (with verapamil) exposure to the drugs are given (mean ± s.e.mean; $n = 6$). Asterisks indicate changes that are statistically significant. The muscles treated with verapamil became inexcitable 10 to 15 min after drug application. The abbreviations are the same as in Table 1.

Discussion

The negative chronotropic effect of naloxone in atria was not unexpected. A similar reduction of the heart rate with comparable concentrations of the drug has already been described in rabbit isolated hearts (Montel & Starke, 1973) and rat atria (Feria *et al.*, 1982). However, an increase of the AP duration induced by naloxone has not been reported before. Most likely, the drug effects in atria and papillary muscles are not caused by a blocking of opioid receptors because (+)-naloxone, which does not bind to opioid receptors (Gayton *et al.*, 1978; Arndt & Freye, 1979), was nearly as effective as the (−)-isomer. A similar, though less prominent, lengthening of the AP duration in papillary muscles was seen with the μ-opioid receptor agonist, morphine. The effective concentration ($120\,\mu mol\,l^{-1}$) was very high, thus making it very unlikely that the effect resulted from activation of opioid receptors. Another agonist, the opioid peptide Met-enkephalin, has no detectable influence on guinea-pig papillary muscles (Iven & Zetler, 1980). One may conclude, therefore, that opioid receptors are not involved in the regulation of the electrical activities of the heart, at least under *in vitro* conditions. Instead, the present results suggest a common, non-specific influence of the structurally-similar molecules, morphine and naloxone, on cardiac cell membranes, an action which is not yet well characterized.

Naloxone is known to decrease the membrane K^+ conductance in frog node of Ranvier (Carratu' & Mitolo-Chieppa, 1982) and in squid axons (Frazier *et al.*, 1973). The prolongation of the terminal repolarization phase by naloxone in atria and papillary muscles is consistent with a reduction of the time-dependent K^+ outward current (Beeler & Reuter, 1977; Ban, 1983). It seems reasonable, therefore, to assume that the drug either decreases the K^+ conductance in these tissues or slows down the activation-inactivation kinetics of the repolarizing K^+ current. At present the influence of naloxone on sinus node action potential is not known. If, however, a similar modification of K^+ current occurred here, it might be an explanation for the negative chronotropic effect of the drug.

In neuronal tissues naloxone causes a prominent reduction of the fast inward Na^+ current (Frazier *et al.*, 1973; Carratu' & Mitolo-Chieppa, 1982). In the heart this effect seems to be minimal, as only a 5–10% reduction of $\dot{V}_{max}$ was observed with the highest drug concentrations. The reactivation kinetics of I_{Na} were apparently unchanged and the prolongation of the functional refractory period is probably caused only by the increase of the AP duration. Naloxone did not alter the slow inward Ca^{2+} current, I_{si}, which explains the lack of an inotropic effect in left atria. In summary, the electrophysiological effect of naloxone in guinea-pig isolated atria and papillary muscles can best be classified as a Class III antiarrhythmic action according to Vaughan Williams (1974). Since (−)-and (+)-naloxone were almost equipotent in all respects, I suggest that the reduction of the K^+ current is caused by a direct influence on the sarcolemma that does not involve stereospecific opioid receptors.

Could such a membrane effect be responsible for the arrhythmogenic and antiarrhythmic actions attributed to naloxone? The doses injected in man (0.2–0.5 mg) are certainly too small to achieve plasma concentrations in the micromolar range. The arrhythmias reported by Pallasch & Gill (1981) are therefore better explained by some central action of the drug. In animal studies, however, the situation is different. Doses up to $10\,mg\,kg^{-1}$ naloxone have been injected intravenously to increase blood pressure during experimental shock (Holaday, 1983) or to prevent arrhythmias (Fagbemi *et al.*, 1982; Bergey & Beil, 1983). This is certainly sufficient to reach plasma concentrations around $5\,\mu g\,ml^{-1}$ for 15–30 min (Ngai *et al.*, 1976; Pace *et al.*, 1979). Comparable concentrations ($2.5–40\,\mu g\,ml^{-1}$) reduced the beating frequency of atria in the present experiments. Thus, a participation of a Class III effect in the antiarrhythmic action of naloxone must be seriously considered. Conclusive evidence, however, can only come from the demonstration of a negative chronotropic or antiarrhythmic influence of (+)-naloxone *in vivo*.

A note of caution may be added. During the work on this paper a series of experiments was performed with a commercial preparation of (−)-naloxone for human use (Narcanti; Du Pont de Nemours, Wilmington, Delaware, U.S.A.). Narcanti consistently shortened AP duration in papillary muscles but increased the functional refractory period, decreased $\dot{V}_{max}$ and delayed the recovery of the Na^+-current from inactivation. This is in clear contrast to the results with pure naloxone described above. The reason is that the ampoules of Narcanti contain methyl paraben $1.8\,mg\,ml^{-1}$ and propyl paraben $0.2\,mg\,ml^{-1}$ (personal information from the manufacturer). Parabens act like local anaesthetics on nerves (Nathan & Sears, 1961) and appropriate concentrations of methyl paraben exactly mimicked the effects of Narcanti in papillary muscles (Brasch, unpublished results). Thus, the apparent Class I antiarrhythmic effects of Narcanti must not be mistaken for genuine effects of naloxone. Parabens have also been responsible for the influence of commercial naloxone preparations on liver cell metabolism (Caldecourt *et al.*, 1983) and on blood vessels (Brandt *et al.*, 1983). Paraben-containing solutions should not be used, therefore, to study the pharmacological effects of naloxone.

I am greatly indebted to Du Pont de Nemours, Pharmaceutical Company, and the National Institute on Drug Abuse for the generous gift of (−)- and (+)-naloxone hydrochloride, respectively. I would also like to thank Mrs G.v. Braun and Mrs E. Obst for skilful technical assistance, Mrs M.-L. Welge for drawing the figures and Mrs G. Mundhenke for the preparation of the manuscript.

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