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Effects of narcotic analgesics and their antagonists on the rabbit isolated heart and its adrenergic nerves

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Summary

1. In isolated perfused hearts of rabbits, the effects of morphine, methadone, pethidine, fentanyl, levallorphan and naloxone on heart rate, the spontaneous outflow of noradrenaline, the uptake of infused noradrenaline and the overflow of noradrenaline in response to stimulation of the accelerans nerves were investigated.

2. At concentrations of 10–100 μM, methadone, fentanyl, levallorphan and naloxone, but not morphine and pethidine, decreased the heart rate. Only pethidine and levallorphan (100 μM) augmented the spontaneous outflow of noradrenaline.

3. With the exception of naloxone, all drugs diminished the neuronal uptake of noradrenaline from the perfusion fluid. Methadone and pethidine (1 μM) were the most effective inhibitors. The inhibitory effect of morphine was not antagonized by naloxone.

4. All drugs increased the overflow of noradrenaline evoked by stimulation of the accelerans nerves at 5 Hz. Simultaneously, the positive chronotropic effect of stimulation was usually enhanced. Morphine also augmented the response to stimulation at 2·5 and 10 Hz. The effect of morphine was prevented by pre-infusion of cocaine. The response to stimulation was never depressed.

5. It is concluded that the adrenergic nerves of the rabbit heart lack specific morphine receptors which in some other sympathetic nerves mediate an inhibition of the stimulation-induced secretion of noradrenaline. The mechanism of the enhancement of adrenergic neurotransmission by relatively high concentrations of the drugs is discussed.

Introduction

Morphine reduces the response to sympathetic nerve stimulation in a variety of organs (Trendelenburg, 1957; Cairnie, Kosterlitz & Taylor, 1961; Szerb, 1961; Gyang, Kosterlitz & Lees, 1964). Henderson, Hughes & Kosterlitz (1972a) and Henderson, Hughes & Thompson (1972b) recently demonstrated that, in the mouse vas deferens and the cat nictitating membrane, morphine decreases the outflow of noradrenaline evoked by field stimulation. The present paper describes the effects of four narcotic analgesics, morphine, methadone, pethidine, fentanyl, the effect of levallorphan, having both agonist and antagonist actions, and that of naloxone, an almost pure antagonist, on the isolated perfused heart of the rabbit and its sympathetic nerves. In particular, the effects of these drugs on the spontaneous outflow of noradrenaline, the overflow evoked by electrical stimulation

of the accelerans nerves and the neuronal uptake of exogenous noradrenaline have been investigated.

Methods

Perfusion of hearts

Rabbits of either sex (1·5–2·5 kg) were stunned by a blow on the head. The hearts were quickly removed and perfused through the coronary vessels at a constant rate of 25 ml/minute. The perfusion fluid contained (mM): NaCl 137; KCl 2·7; $CaCl_2$ 2·0; $MgCl_2$ 1·1; $NaHCO_3$ 12·0; NaH_2PO_4 0·4; glucose 5; ascorbic acid 0·06; disodium-edetate 0·03. It was saturated with a mixture of 95% O_2 and 5% CO_2 and warmed to 37° C. A thread was tied to the apex of the heart and connected to a strain gauge (Hugo Sachs Elektronik, model K 80). Diastolic tension was adjusted to 10 grams. The contractions were used to trigger a rate-meter (Eka-Puls, Hugo Sachs Elektronik). Tension and ventricular rate were displayed on a Hellige recorder model HE 17. Experimental procedures were started 30 min after the preparation had been set up.

Removal of infused noradrenaline

The coronary vessels were perfused for 10 min with (−)-noradrenaline 0·06 μM. The venous effluent was collected for determination of the amine. The amount of noradrenaline removed from the perfusion fluid during the passage through the heart was calculated as % of the amount infused. Drugs were infused 10 min before and during the infusion of noradrenaline.

Sympathetic nerve stimulation

The hearts were prepared with their postganglionic sympathetic nerves intact (Huković & Muscholl, 1962). The nerves were pulled through platinum ring electrodes. Rectangular pulses of 3 ms duration and a constant current strength of 8 mA (supramaximal) were delivered from a Stimulator II (Hugo Sachs Elektronik). During the three or four stimulation periods of each experiment (S_1–S_4), the right and left nerves were stimulated alternately, each side twice for 15 seconds. Thus, stimulation periods lasted for 1 min; they were separated by intervals of 14 minutes. Unless otherwise stated, the stimulation frequency was 5 Hz. The perfusate was collected during, and for 1 min after, stimulation for the determination of noradrenaline. In preliminary experiments it was found that at least 85% of the total stimulation-induced overflow of noradrenaline was collected in this 2-min period. The first stimulation was always applied before the addition of any drug. In general, the responses to S_2, S_3 or S_4, in the presence of drugs, were expressed as % of the response to S_1.

Determination of noradrenaline

Noradrenaline was adsorbed on Al_2O_3 at pH 8·5. After elution with 0·12 N HCl it was determined fluorimetrically according to the trihydroxyindole procedure as modified by Palmer (1964). None of the drugs investigated, except methadone, interfered with the assay method; this restricted the use of this drug to concentrations below 100 μM.

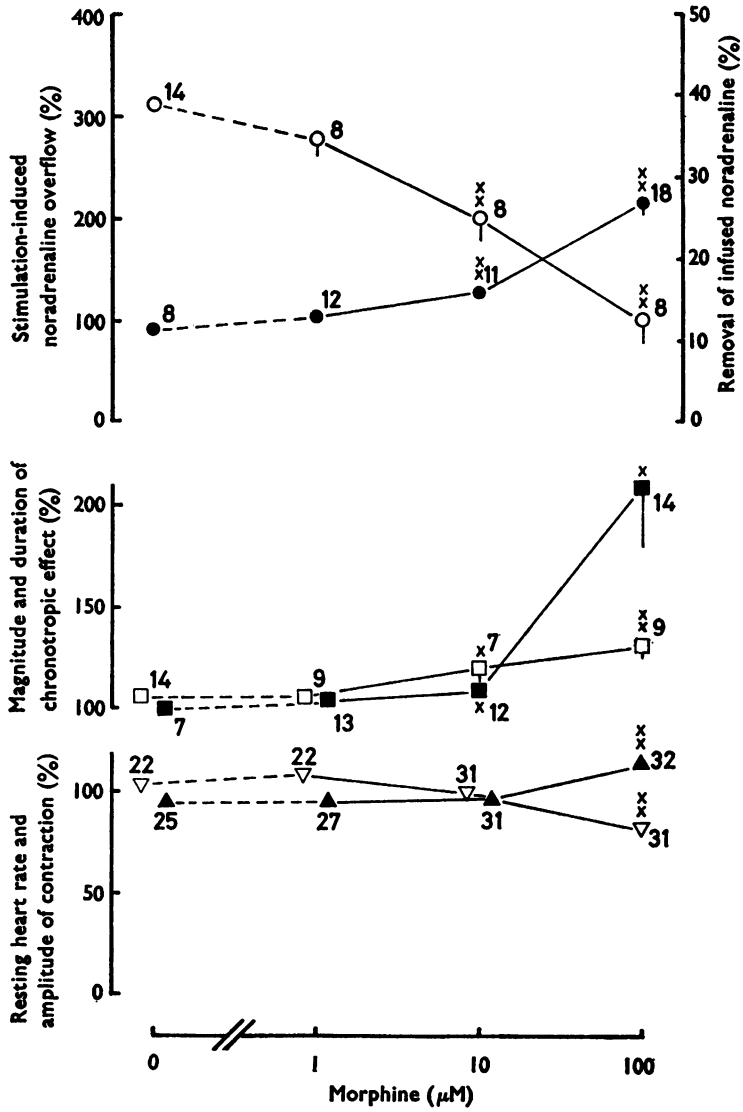

FIG. 1. Influence of morphine on the rabbit isolated heart and its sympathetic nerves. Resting rate (▲) and amplitude (▽) of contraction 10 min after the start of the infusion of morphine (or in Figs. 2–5, a related drug) are expressed as % of the values before the infusion. Positive chronotropic effect of infused noradrenaline, 0·06 μM (□); the maximal heart rate attained is expressed as % of the heart rate before the infusion. Removal of infused noradrenaline (○); the amount removed during the passage through the coronary vessels is expressed as % of the amount infused. Duration of the positive chronotropic effect of stimulation of the right and left accelerans nerves (3 ms, 8 mA, 5 Hz, each nerve twice for 15 s in 1 min) (■), expressed as time from the onset of stimulation until the increase in heart rate had decayed by 50%. The duration of the response to the second stimulation period (S_2), in the presence of morphine, is expressed as % of the duration of the pre-drug response to the first stimulation period (S_1). Stimulation-induced overflow of noradrenaline (●); the overflow evoked by S_2 is expressed as % of that evoked by S_1. The points indicate means and vertical bars S.E. mean; the numbers indicate the numbers of experiments. Significant differences from controls: * $P<0·05$; ** $P<0·001$.

Experiments with [^3H]-noradrenaline

(−)-[^{3}H]-noradrenaline, specific activity 1·6 Ci/mmol, was infused through the aortic cannula for 15 min to give a final arterial concentration of 0·1 μM. The perfusate was collected in 2 min samples from 30 to 70 min after the end of the infusion of [^{3}H]-noradrenaline. Drugs were given from 50 to 70 min after the end of this infusion. The tritiated material in the venous effluent was determined by liquid scintillation counting.

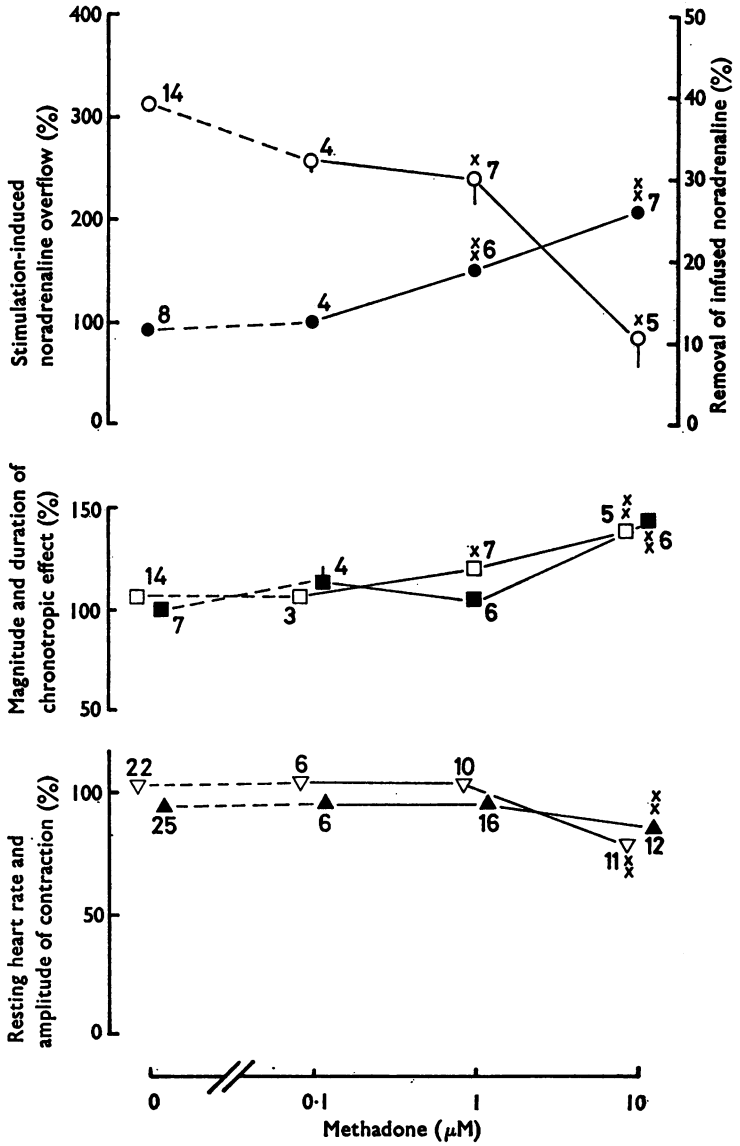

FIG. 2. Influence of methadone on the rabbit isolated heart and its sympathetic nerves. For explanation, see legend to Figure 1.

Drugs

(−)-noradrenaline base, (−)-methadone hydrochloride, pethidine hydrochloride (Farbwerke Hoechst); morphine hydrochloride, cocaine hydrochloride (Merck); fentanyl citrate (Janssen); levallorphan tartrate (Hoffman-La Roche); naloxone hydrochloride (Endo Laboratories); (−)-[7-^{3}H]-noradrenaline (Amersham-Buchler). All concentrations are expressed as μM base.

Statistical analysis

Means ± S.E. mean are given throughout this paper. Significance of differences was calculated with Student's *t*-test.

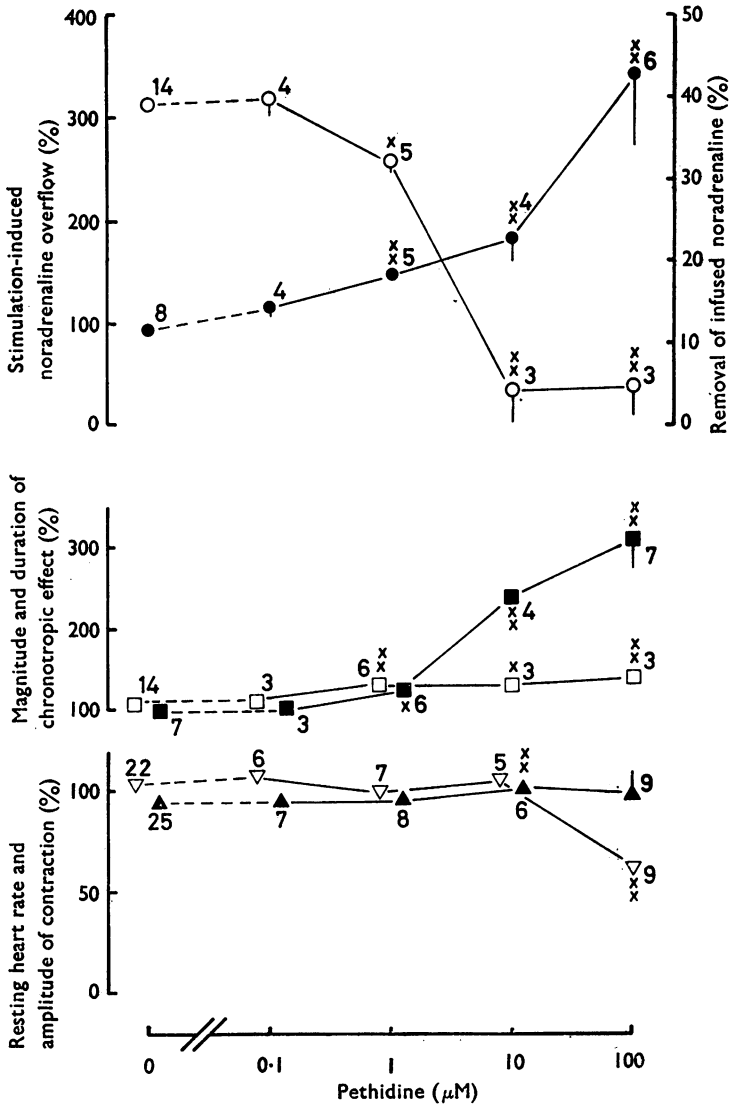

FIG. 3. Influence of pethidine on the rabbit isolated heart and its sympathetic nerves. For explanation, see legend to Figure 1.

Results

Effects on the resting amplitude of contraction, resting heart rate, and spontaneous outflow of noradrenaline

The amplitude of the contractions in the absence of nerve stimulation, 30 min after the preparation had been set up, was within the range of 7 to 13 g, and without the addition of drugs remained approximately constant for 50 minutes. At concentrations of 10–100 μM, all drugs depressed the contraction amplitude (Figures 1–5). The resting heart rate varied between 80 and 150/minute. In experiments without addition of drugs, it declined slowly. Methadone (Fig. 2),

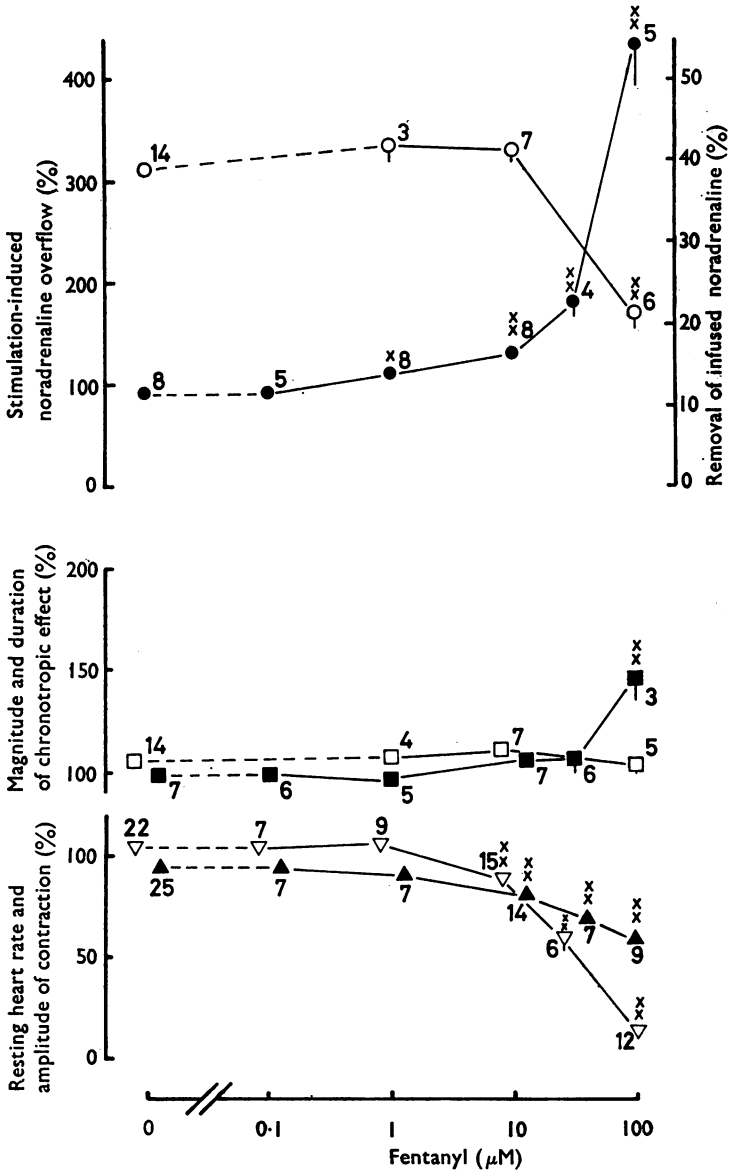

FIG. 4. Influence of fentanyl on the rabbit isolated heart and its sympathetic nerves. For explanation, see legend to Figure 1.

fentanyl (Fig. 4), levallorphan (10–100 μM ; not shown) and naloxone (Fig. 5) decreased the heart rate. In contrast, morphine (100 μM ; Fig. 1) and pethidine (10 μM ; Fig. 3) had a significant positive chronotropic effect, but their influence on the heart rate was somewhat variable.

The resting outflow of noradrenaline, 30 min after the preparations had been set up, was $2{\cdot}6 \pm 0{\cdot}5$ ng/min ($n=9$). Of the drugs investigated, only pethidine (100 μM) and levallorphan (100 μM) significantly changed the resting outflow ($4{\cdot}9 \pm 0{\cdot}8$ ng/min, $n=3$, and $4{\cdot}6 \pm 0{\cdot}5$ ng/min, $n=4$, respectively ; $P<0{\cdot}05$). The value in the presence of morphine (100 μM) was $3{\cdot}7 \pm 0{\cdot}3$ ng/min ($n=12$, NS).

For the determination of the resting outflow of noradrenaline and its metabolites, the hearts were pre-infused with (−)-[^{3}H]-noradrenaline, as described in

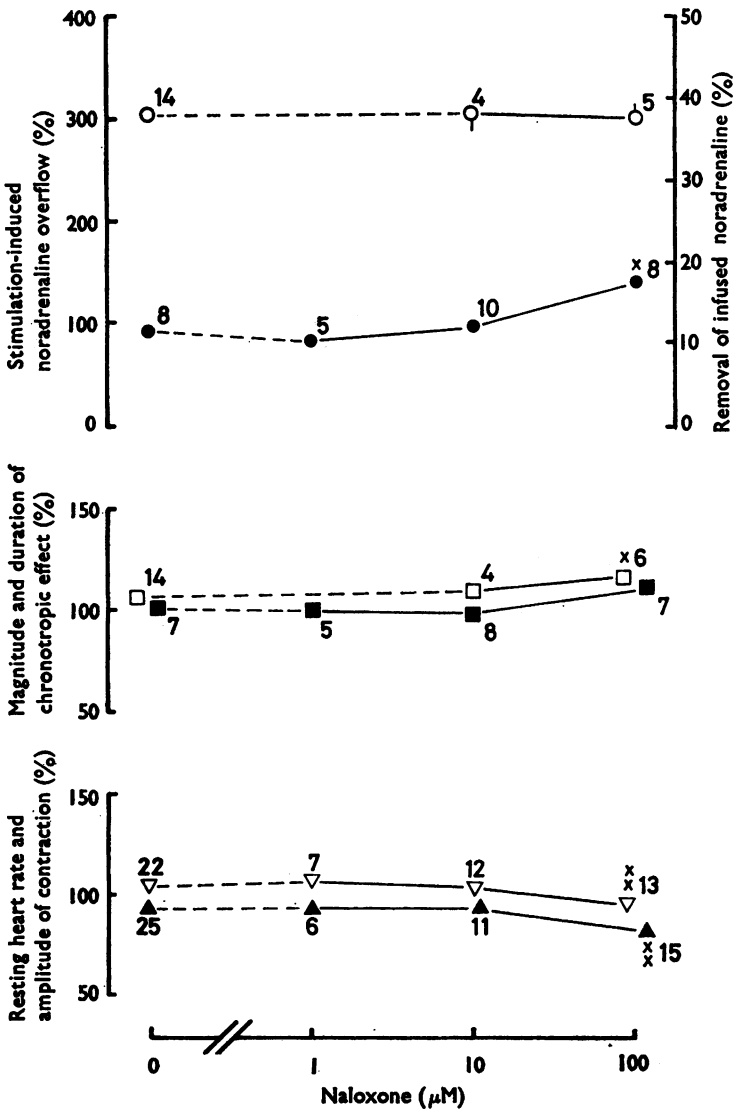

FIG. 5. Influence of naloxone on the rabbit isolated heart and its sympathetic nerves. For explanation, see legend to Figure 1.

Methods. In confirmation of previous work (Starke, 1971), the late component of the efflux of tritiated material, 30–70 min after the end of the infusion, followed a single exponential course with a rate constant of 0·012/minute. The effects of some narcotic analgesics and antagonists are illustrated in Figure 6. Morphine and pethidine caused a concentration-dependent increase of the outflow of tritiated compounds; 1 μM morphine had no influence. The effect of levallorphan was greater than that of morphine and pethidine, whereas naloxone accelerated the outflow of tritiated compounds only slightly at the high concentration of 100 μM.

Effects on the removal and the positive chronotropic action of exogenous noradrenaline

In control experiments, 38·0 ± 1·5% (n=14) of the infused noradrenaline was removed from the fluid during the passage through the heart. The removal and, therefore, the neuronal uptake was diminished by all narcotic analgesics (Figs. 1–4) and by levallorphan (100 μM), but not by naloxone (Figure 5).

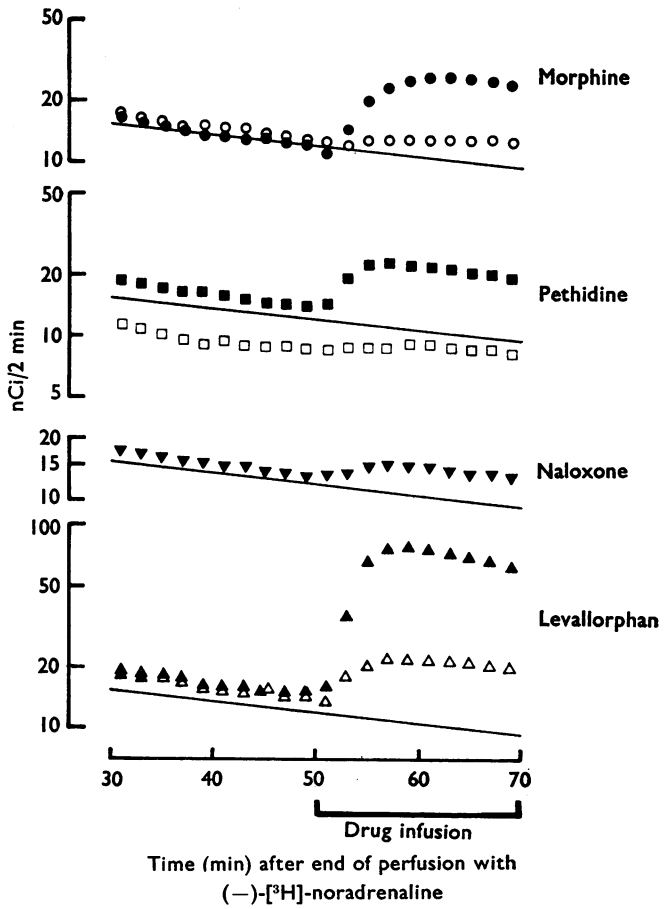

FIG. 6. Effect of narcotic analgesics and antagonists on the outflow of tritiated compounds from rabbit hearts pre-perfused with 0·1 μM (−)-[^{3}H]-noradrenaline. From 30 to 70 min after the end of this perfusion, the outflow of tritiated compounds from control hearts followed a single exponential course (straight lines, calculated by regression analysis from 4 expts). Drugs were infused as indicated at concentrations of 10 μM (open symbols) or 100 μM (closed symbols). The values are the means of 3 expts. The standard errors were 4–19% of the corresponding means.

The heart rate was increased by 0·06 μM noradrenaline to 106·5 ± 2·1% (n=14). In most experiments, the concentrations of the drugs that inhibited the removal of noradrenaline also enhanced its positive chronotropic effect, with the exceptions of 100 μM fentanyl and 100 μM naloxone (Figures 1–5).

Effects on the overflow of noradrenaline and the increase in heart rate caused by sympathetic nerve stimulation

When the accelerans nerves were stimulated, the outflow of noradrenaline increased. The overflow caused by the first stimulation period (S_1, in the absence of drugs) amounted to 55·0 ± 4·8 ng (n=169, all experiments at 5 Hz combined). In experiments without drugs, the overflow caused by S_2 was 91·0 ± 6·5% of that caused by S_1 (n=8). Morphine, methadone and pethidine at concentrations which inhibited the uptake of noradrenaline augmented the stimulation-induced overflow (Figures 1–3). Fentanyl (Fig. 4), levallorphan (10 μM) and naloxone (Fig. 5) caused a slight increase at concentrations which did not interfere with amine uptake. Fentanyl (100 μM) reduced the uptake of noradrenaline only by one half, but enhanced the stimulation-induced overflow 5-fold, i.e. much more than morphine, methadone and pethidine at concentrations producing a greater inhibition of uptake (100, 10 and 10 μM, respectively). Levallorphan increased the stimulation-induced overflow at a concentration of 10 μM, but decreased it at a concentration of 100 μM, possibly by a local anaesthetic block of impulse conduction.

The influence of morphine on the overflow of noradrenaline evoked by stimulation at various frequencies is presented in Table 1. The mean values of overflow in response to stimulation in the absence of morphine (S_1) were 29·5 ± 2·2 ng (n=33), 60·3 ± 10·6 ng (n=49) and 152·6 ± 10·9 ng (n=16) at stimulation frequencies of 2·5, 5 and 10 Hz, respectively. At concentrations of 0·1 and 1 μM, morphine had no influence. Higher concentrations increased the response to any of the frequencies used.

TABLE 1. *Effect of morphine on the overflow of noradrenaline evoked by electrical stimulation at different frequencies*

Morphine (μM)	Stimulation frequency (Hz)	Noradrenaline overflow due to S_2 (% of S_1)	
0	2·5	90·6 ± 5·6	(5)
	5	91·0 ± 6·5	(8)
	10	80·3 ± 5·9	(8)
0·1	2·5	96·1 ± 7·0	(7)
1	2·5	106·3 ± 5·8	(7)
	5	103·5 ± 5·1	(12)
10	2·5	117·5 ± 7·3*	(9)
	5	127·3 ± 5·1†	(11)
	10	138·5 ± 6·1†	(5)
100	2·5	208·4 ± 24·6*	(5)
	5	217·1 ± 12·2†	(18)
	10	222·0 ± 28·4†	(3)

In each experiment there were two stimulation periods (S_1, S_2) with an interval of 14 minutes. The overflow of noradrenaline caused by S_2 was expressed as % of that caused by S_1. The values are the mean overflows ± S.E. mean; numbers of experiments are given in brackets. Morphine infusion was started 10 min before and lasted until 5 min after the onset of S_2. Significant differences from controls (morphine=0): *P<0·05; †P<0·001.

In control experiments, the cardiac acceleration caused by successive stimulation periods was approximately constant. The narcotic analgesics and the antagonists tended to prolong rather than increase the response to stimulation. This prolongation roughly paralleled the augmentation of the stimulation-induced overflow of noradrenaline (Figures 1–5). There were, however, exceptions. Thus, the very large increase of overflow caused by 100 μM fentanyl was accompanied by only a small increase in the duration of the positive chronotropic effect.

In some experiments, drug infusions were stopped 5 min after the onset of S_2, and further stimulations (S_3, S_4) applied during perfusion with drug-free solution. The stimulation-induced overflow of noradrenaline was still higher ($P<0{\cdot}05$) 10 or 25 min after the end of the infusion of morphine (100 μM) than in experiments without drugs; it amounted to $203{\cdot}4\pm9{\cdot}9\%$ ($n=11$) or $200{\cdot}1\pm36{\cdot}2\%$ ($n=4$) of the overflow caused by S_1. Similar results were obtained at a stimulation frequency of 10 Hz, and with 10 μM morphine. In contrast, 10 min after the end of the infusion of the other drugs, the response to stimulation was not significantly different from controls.

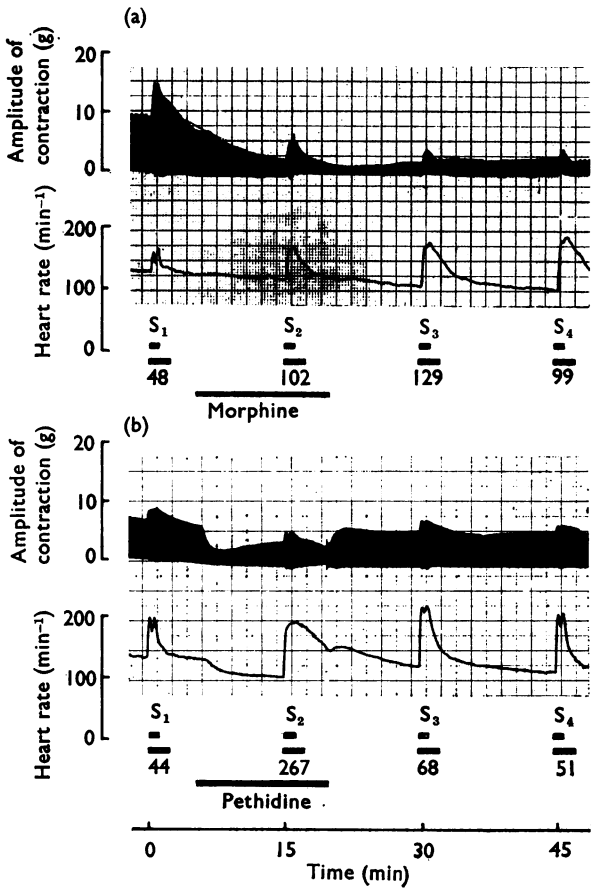

FIG. 7. Influence of morphine (a) and pethidine (b) on rabbit isolated hearts and their responses to sympathetic nerve stimulation. In each experiment, 4 stimulation periods were applied (S_1 to S_4). In a and b the upper tracing indicates tension (g) of contraction and the lower tracing the heart rate (beats/minute). Horizontal bars: the uppermost indicate duration of stimulation; the middle bars the duration of collection of effluent together with numbers indicating the amount of noradrenaline (ng) overflowing in 2 min; the lowest bars indicate duration of infusion of morphine or pethidine, both 100 μM.

Typical experiments demonstrating the effects of morphine and pethidine are shown in Figure 7. Morphine and pethidine reduced the amplitude of contraction, and pethidine decreased the resting heart rate. Either drug enhanced the increase of heart rate and the overflow of noradrenaline evoked by sympathetic nerve stimulation (S_2). The influence of morphine on the resting amplitude of contraction and the response to stimulation was maintained throughout the washout period. On the other hand, the effect of pethidine on resting amplitude and heart rate was reversed by washing, and the response to stimulation rapidly returned to the pre-infusion level.

Interaction of cocaine and morphine

In some experiments, the effect of morphine on the stimulation-induced overflow of noradrenaline was tested, after the neuronal uptake system of noradrenaline had been blocked by cocaine (Table 2). Cocaine (S_2) increased the response to stimulation. In the presence of cocaine, the additional infusion of morphine (S_3) did not cause any significant change.

TABLE 2. *Interaction of cocaine and morphine on the stimulation-induced overflow of noradrenaline*

Group	Drug concentrations	Stimulation frequency (Hz)	Stimulation-induced noradrenaline overflow (ng) S_1	S_2	S_3
1	Cocaine, 20 μM	2·5	28·4± 4·2	50·4±11·4	48·4± 7·2 (5
		5	79·3±24·5	130·5±40·6	133·5±44·5 (8)
2	Cocaine, 20 μM+ morphine, 1 μM	2·5	30·5± 3·7	61·1± 9·6	69·6± 8·7 (5)
3	Cocaine, 20 μM+ morphine, 10 μM	5	60·6±15·9	90·9±26·4	101·1±29·1 (5)
4	Cocaine, 20 μM+ morphine, 100 μM	5	58·8±16·3	90·0±17·0	121·8±31·8 (5)

In each experiment, three stimulation periods were applied (S_1 to S_3). Cocaine infusion was started 10 min before S_2, and, in groups 2 to 4, infusion of morphine was begun 10 min before S_3; both infusions were continued until the end of the experiment. The values are the means ±S.E. mean; the numbers of experiments are given in brackets.

Interaction of naloxone and morphine

Experiments were performed to test whether naloxone counteracts the inhibition of the uptake of noradrenaline by morphine. In the presence of morphine (10 μM), the removal of infused noradrenaline from the perfusion medium was reduced to 25·1 ±2·2% (n=8, cf. Figure 1). In the presence of both morphine (10 μM) and naloxone (100 μM), the removal of the amine was further diminished to 16·1 ±3·4% (n=6, $P<0·05$). Thus, naloxone did not antagonize but increased the inhibitory effect of morphine.

Discussion

With the exception of naloxone, all the drugs investigated inhibited the removal of infused noradrenaline from the fluid perfusing the heart. At the concentration used (0·06 μM), noradrenaline is mainly removed by uptake into the sympathetic cardiac nerves (Lindmar & Muscholl, 1964); thus, morphine, methadone, pethidine, fentanyl and levallorphan block the noradrenaline transport system of the neuronal membrane. Dengler & Titus (1961) have previously reported that morphine reduces the binding of noradrenaline by a variety of cat tissues. High concentrations of narcotic analgesics and antagonists inhibit the uptake of the amine into

synaptosomes from the rabbit brain (Ciafalo, 1972). There was no correlation between the ability of the drugs to inhibit the uptake of noradrenaline into the heart and their reported analgesic efficacy (for references, see Herz & Teschemacher, 1971). Thus, the potent analgesic, fentanyl, inhibited the uptake of noradrenaline only at a concentration of 100 μM ; on the other hand, the weak analgesic pethidine decreased it at a concentration of 1 μM. Moreover, levallorphan, which has agonist and antagonist actions, also interfered with the binding of noradrenaline ; naloxone did not antagonize but slightly enhanced the inhibitory effect of morphine. The effects on noradrenaline uptake can therefore not be due to an interaction with a specific morphine receptor site.

An increase of the positive chronotropic effect of noradrenaline and sympathetic nerve stimulation usually accompanied the inhibition of uptake. Methadone and pethidine inhibited amine uptake at a concentration (1 μM) which may occur in human plasma during therapy (Inturrisi & Verebely, 1972). This cocaine-like effect will tend to counteract the circulatory depression evoked by the action of these drugs on the central nervous system or the vascular and myocardial muscles.

The spontaneous outflow of noradrenaline was not influenced by low concentrations of the drugs but was increased by 100 μM of pethidine, levallorphan and probably also morphine. At this concentration, these drugs inhibit the uptake of noradrenaline across the neuronal membrane ; thus, the increase of outflow may be accounted for by a blockade of the re-uptake of spontaneously released transmitter. A blockade of re-uptake, and the ensuing increase of the biophase concentration of noradrenaline, may also be responsible for the positive chronotropic effect of morphine (100 μM) and pethidine (10 μM). Both a 'direct' negative chronotropic action and an increase of the concentration of noradrenaline in the vicinity of its receptors probably contribute to the effect on heart rate of most of the agents tested (cf. Trendelenburg, 1968).

Morphine, pethidine, levallorphan and naloxone increased the outflow of tritiated noradrenaline and metabolites from hearts pre-perfused with (−)-[^{3}H]-noradrenaline. At the time the drugs were added, the efflux of tritiated compounds largely originates from the adrenergic nerves (Starke, 1971). The increase can in part be accounted for by an inhibition of the re-uptake of spontaneously liberated [^{3}H]-noradrenaline. However, efflux of labelled compounds was also accelerated by naloxone (100 μM), which does not interfere with the neuronal uptake mechanism ; moreover, the relative efficiencies of morphine, pethidine and levallorphan in augmenting the efflux of labelled compounds on the one hand, and in inhibiting the uptake of noradrenaline on the other, were quite different. Presumably intraneuronal actions may contribute to the increase of the efflux rate.

All of the drugs used increased the overflow of noradrenaline and the rise of heart rate in response to sympathetic nerve stimulation. There was no correlation between the analgesic or antagonist activities of the drugs and their ability to enhance the response to stimulation. At concentrations which enhanced the stimulation-induced overflow, morphine, methadone, and pethidine blocked the uptake of noradrenaline. This inhibition of re-uptake probably accounts for the increase of overflow during nerve stimulation. This view is favoured by the interaction of cocaine and morphine. After uptake had been blocked, and the stimulation-induced overflow increased by cocaine, the effect of morphine was lost.

Fentanyl, levallorphan, and naloxone enhanced the overflow of noradrenaline in response to stimulation at concentrations which did not significantly impair its neuronal uptake. No attempt was made to decide whether these drugs interfere with the extraneuronal binding or metabolic degradation of noradrenaline, or facilitate the liberation of the transmitter.

In a variety of organs, narcotic analgesic drugs in low concentrations reduce the response to sympathetic nerve stimulation, probably by a depression of transmitter release ; antagonists counteract this inhibition (Trendelenburg, 1957 ; Cairnie *et al.*, 1961 ; Szerb, 1961 ; Gyang *et al.*, 1964 ; Henderson *et al.*, 1972a, b). In the sinoatrial node-right atrial preparation of the rabbit, Kennedy & West (1967) showed that morphine, in the presence of atropine, does not decrease the positive chronotropic effect of intranodal adrenergic nerve stimulation. Our experiments confirm their results and also demonstrate that three other narcotic analgesics are devoid of any inhibitory action on the cardiac response to sympathetic nerve stimulation ; the stimulation-induced overflow and, presumably, the release from the nerve terminals of the adrenergic transmitter are not impaired.

The inhibitory effect of morphine on adrenergic neurotransmission is greater at low than at high frequencies of stimulation (Cairnie *et al.*, 1961). In our experiments, morphine did not reduce the response to stimulation in the range between 2·5 and 10 Hz. It might be supposed that narcotic analgesics inhibited the release of noradrenaline from the accelerans nerves of the rabbit and that this inhibition was masked by a simultaneous blockade of noradrenaline re-uptake. However, the narcotic analgesics failed to impair neurotransmission in concentrations far below those interfering with the neuronal uptake mechanism. Moreover, morphine did not inhibit the effect of stimulation even after the uptake of noradrenaline had been blocked by cocaine. Thus, the adrenergic nerves of the rabbit heart, like those of the cat heart (Cairnie *et al.*, 1961) and the guinea-pig myenteric plexus (Henderson *et al.*, 1972b) are insensitive to the depressant effect of narcotic analgesics. It is not known whether the central noradrenergic neurones belong to the type responding with an inhibition, or to the type, described in this paper, responding ' unspecifically ' to relatively high concentrations with an enhancement of neurotransmission.

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