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Peripheral sympatho-inhibitory cardiovascular effects of opioid peptides in anaesthetized rabbits

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1 Opioid agonists influence isolated cardiovascular tissues from rabbits, as well as the cardiovascular system of pithed rabbits, through presynaptic receptors on postganglionic sympathetic nerve fibres. The present experiments were carried out in order to study effects which result from activation of these receptors in anaesthetized rabbits.

2 In pithed rabbits with electrically stimulated sympathetic outflow, infusion of [D-Ala^2-D-Leu^5]-enkephalin (DADLE) $10\,\mu g\,kg^{-1}\,min^{-1}$ and dynorphin-(1–13) (dynorphin) $1\,\mu g\,kg^{-1}\,min^{-1}$ decreased the plasma noradrenaline concentration, mean arterial pressure (MAP) and heart rate. The effects of dynorphin and, less completely, those of DADLE were antagonized by the peripherally selective opioid antagonists N-methyl naloxone bromide (NMN) $1.3\,mg\,kg^{-1}$ and N-methyl levallorphan methanesulphonate (NML) $1–3\,mg\,kg^{-1}$.

3 In pentobarbitone-anaesthetized rabbits, DADLE $3–30\,\mu g\,kg^{-1}\,min^{-1}$ and dynorphin $0.3–3\,\mu g\,kg^{-1}\,min^{-1}$ decreased the plasma noradrenaline concentration and MAP. The highest dose of dynorphin also decreased heart rate, whereas DADLE $10\,\mu g\,kg^{-1}\,min^{-1}$ caused slight cardioacceleration. The effects of DADLE but not those of dynorphin decreased upon repeated administration.

4 The effects of dynorphin $10\,\mu g\,kg^{-1}\,min^{-1}$ were abolished or greatly attenuated by NMN $1.3\,mg\,kg^{-1}$ and NML $3\,mg\,kg^{-1}$. In contrast, the antagonists reduced only slightly the blood pressure-lowering effect of DADLE $10\,\mu g\,kg^{-1}\,min^{-1}$ and did not reduce significantly the effects of DADLE on the plasma noradrenaline level and heart rate.

5 It was concluded that systemically administered dynorphin produces sympatho-inhibition and an ensuing fall in blood pressure by an action at peripheral receptors, in all probability presynaptic κ-receptors on postganglionic sympathetic nerve fibres. The effects of DADLE are more complex and may involve both central and peripheral components.

Introduction

Activation of presynaptic opioid receptors on postganglionic sympathetic nerve fibres decreases sympathetic neurotransmitter release and, consequently, the response to sympathetic nerve impulses in isolated cardiovascular tissues of the rabbit (ear artery: Knoll, 1976; Illes *et al.*, 1985; ileocolic artery: von Kügelgen *et al.*, 1985; jejunal arteries: Illes *et al.*, 1986; Ramme *et al.*, 1986; pulmonary artery: Seelhorst & Starke, 1986; portal vein: Szabo *et al.*, 1987; heart: Starke *et al.*, 1985). Similar results have been obtained in other species (guinea-pig atria: Ledda *et al.*, 1984; Fuder *et al.*, 1986; rat tail artery: Illes *et al.*, 1987; cat spleen: Gaddis & Dixon, 1982). We have previously shown that these peripheral presynaptic opioid receptors can also be activated in pithed rabbits with electrically stimulated sympathetic outflow: both the release of noradrenaline and the arterial blood pressure decreased upon infusion of opioids (Ensinger *et al.*, 1984; 1986; Szabo *et al.*, 1986). In the rabbit, the receptors were exclusively of the δ- and κ-type.

Opioids can influence cardiovascular function at several other levels in addition to peripheral presynaptic opioid receptors. They can initiate a depressor reflex at pulmonary receptors; the afferent fibres run in the vagus nerve (Willette *et al.*, 1982). They influence the cardiovascular centres directly; the effects depend on the site of administration and on the

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agonist used (e.g., Feuerstein & Faden, 1982; Carter & Lightman, 1985; Punnen & Sapru, 1986). Opioids can also affect ganglionic transmission (Konishi *et al.*, 1979) and cardiovascular effector cells (heart: Ruth & Eiden, 1984; Gautret & Schmitt, 1985; vascular smooth muscle: Altura *et al.*, 1984; Ruth *et al.*, 1984).

Which of these various sites come into play when opioids are administered systemically to living animals? Evidence has been obtained for an *in vivo* action at pulmonary receptors (Willette *et al.*, 1982), at central sites (Gautret & Schmitt, 1985; Rhee *et al.*, 1985), and the sinus node (Gautret & Schmitt, 1985). To our knowledge, an activation of peripheral ganglionic receptors or presynaptic opioid receptors of cardiovascular sympathetic neurones has never been demonstrated *in vivo*. The present study addresses this problem. The experiments were carried out in anaesthetized rabbits. First, we measured the effects of [D-Ala^2-D-Leu^5]enkephalin (DADLE) and dynorphin-(1–13) (dynorphin) on the plasma noradrenaline levels, arterial blood pressure and heart rate. Second, the possibility of a peripheral component was examined using the peripherally selective opioid antagonists N-methyl naloxone bromide (NMN) (Giles *et al.*, 1983) and N-methyl levallorphan methanesulphonate (NML) (Dragonetti *et al.*, 1983).

Methods

Preparation

Rabbits of either sex (1.8–3.2 kg) were used in two models. In both models the rabbits were given 10 ml of Rheomacrodex (5 g Dextran 40 per 100 ml 0.9% NaCl) at the end of surgery in order to compensate for blood loss; moreover, 400–500 u of heparin-sodium was given in order to prevent blood clotting in the cannulae. Blood pressure and heart rate were recorded with a Statham P23 Db pressure transducer connected to a pen recorder with a built-in integrator. Mean arterial pressure (MAP) was calculated as $2/3 \times$ diastolic pressure $+ 1/3 \times$ systolic pressure.

Pithed rabbits with electrically stimulated sympathetic outflow Rabbits were anaesthetized with sodium pentobarbitone 60–80 mg kg^{-1} i.v. The trachea was cannulated and artificial respiration at 40–50 cycles min^{-1} commenced. Left and right carotid arteries and jugular veins were cannulated with polyethylene tubing. One artery served for blood pressure measurement, the other for blood sampling. Veins served for drug administration. Gallamine triethiodide 5 mg kg^{-1} was given i.v. in order to block skeletal muscle contractions caused by pithing and electrical stimulation. A hole was made in the parietal bone, at the midline of the skull, and a non-insulated metal rod (diameter 3.5 mm) was inserted approximately 25 cm down the spinal canal as measured from the hole. The metal rod destroyed the spinal cord and also served to stimulate electrically the peripheral sympathetic nerves. A 10 cm length of copper wire inserted subcutaneously between the scapulae served as the indifferent electrode. In order to destroy fully the animal's brain and to stop bleeding, the skull was firmly stuffed with soft paper. Continuous electrical stimulation was carried out at a frequency of 2 Hz with 0.3 ms square-wave pulses at a current strength of 140 mA.

Anaesthetized rabbits Rabbits were anaesthetized with sodium pentobarbitone 60–80 mg kg^{-1} i.v. The trachea was cannulated and artificial respiration at 40–50 cycles min^{-1} commenced. Left and right femoral arteries and veins were cannulated with polyethylene tubing. One artery served for blood pressure measurement, the other for blood sampling. Veins served for drug administration. An infusion of pentobarbitone (20 mg kg^{-1} h^{-1} i.v.) was started at the end of surgery in order to maintain anaesthesia.

Determination of plasma noradrenaline

The concentration of noradrenaline in the plasma was estimated from 2 ml blood samples by alumina chromatography followed by high pressure liquid chromatography and electrochemical detection as previously described (Majewski *et al.*, 1982). The recovery of noradrenaline was $70.1 \pm 0.9\%$ ($n = 198$).

Experimental design

Physiological saline, DADLE or dynorphin was infused either once for 5 min beginning at $t = 50$ min ($t = 0$ min being the end of surgery), or four times for 5 min each, the infusion periods beginning at $t = 50$ (P1), $t = 80$ (P2), $t = 110$ (P3) and $t = 140$ min (P4). Blood samples were taken 1 min before infusion periods and at the end of infusion periods. Blood pressure and heart rate were also evaluated at these time points. Plasma noradrenaline, blood pressure and heart rate values at the end of an infusion period were expressed as a percentage of the values 1 min before the respective infusion period.

NMN or NML, when used, was injected as a bolus at $t = 45$ and $t = 40$ min, respectively, i.e. 5 or

Table 1 Plasma noradrenaline concentration, mean arterial pressure (MAP) and heart rate before infusion of physiological saline, [D-Ala^2-D-Leu^5]enkephalin (DADLE) or dynorphin

			Pretreatment		
		Control	*NMN* (1.3 mg kg^{-1})	*NML* (1 mg kg^{-1})	*NML* (3 mg kg^{-1})
Plasma noradrenaline (pg ml^{-1})	Pithed rabbits	833 ± 108 (26)	704 ± 93 (24)	777 ± 71 (25)	670 ± 79 (24)
	Anaesthetized rabbits	558 ± 73 (79)	595 ± 97 (23)		**1013 ± 263 (23)
MAP (mmHg)	Pithed rabbits	61.6 ± 1.8	62.9 ± 2.9	58.9 ± 2.6	63.8 ± 2.3
	Anaesthetized rabbits	72.1 ± 1.9	78.4 ± 3.0		*79.6 ± 3.1
Heart rate (beats min^{-1})	Pithed rabbits	227 ± 10	229 ± 10	234 ± 9	229 ± 10
	Anaesthetized rabbits	265 ± 5	273 ± 11		275 ± 11

In pithed rabbits, the sympathetic outflow was stimulated electrically at 2 Hz. Animals were either not pretreated or were pretreated with N-methyl-naloxone (NMN) 1.3 mg kg^{-1} at $t = 45$ min or with N-methyl levallorphan (NML) 1 or 3 mg kg^{-1} at $t = 40$ min. Values were measured at $t = 49$ min. Means ± s.e.mean of n (number in parentheses) experiments. n also refers to the MAP and heart rate values. Significant differences from respective control: *$P < 0.05$; **$P < 0.01$.

10 min before the start of the infusion of physiological saline, DADLE or dynorphin.

Statistical evaluation

Means ± s.e.mean are given throughout. Outlying observations were deleted using Grubbs' test (Grubbs & Beck, 1972). The Mann-Whitney test was used to test differences between groups for significance (Snedecor & Cochran, 1967). $P < 0.05$ was taken as the limit of significance.

Drugs

[D-Ala^2-D-Leu^5]enkephalin (DADLE) was obtained from Bachem (Bubendorff, Switzerland), dynorphin-(1–13) (dynorphin) and gallamine triethiodide from Sigma (München, F.R.G.), N-methyl naloxone bromide (NMN) from Boehringer Ingelheim (Ingelheim, F.R.G.), N-methyl levallorphan methanesulphonate (SR 58002C; abbreviated here NML) from Sanofi Research Center (Milano, Italy). Doses refer to the salts. Infusions of DADLE and dynorphin were always freshly prepared from frozen stock solutions. All drugs were administered intravenously. Drug infusions were at a rate of 22.8 ml h^{-1}. Bolus injections had a volume of 1 ml kg^{-1} body weight.

Results

Experiments in pithed rabbits

Initial experiments were carried out in pithed rabbits with electrically stimulated sympathetic outflow in order to find effective doses of NMN and NML. Plasma noradrenaline levels, MAP and heart rate at $t = 49$ min after surgery, i.e., 1 min before administration of opioid peptides or physiological saline, are shown in Table 1. The values are similar to those obtained previously (Ensinger *et al.*, 1986). The opioid antagonists NMN 1.3 mg kg^{-1} and NML 1 or 3 mg kg^{-1}, when injected intravenously at $t = 45$ and $t = 40$ min, respectively, caused transient hypotension. However, Table 1 shows that none of the parameters measured were changed at $t = 49$ min.

DADLE 10 μg kg^{-1} min^{-1} and dynorphin 1 μg kg^{-1} min^{-1} were infused for 5 min, beginning at $t = 50$ min. As shown in Figure 1, the peptides significantly decreased the plasma noradrenaline level, MAP and (dynorphin only) heart rate. NMN and either dose of NML abolished the effects of dynorphin. The effects of DADLE were antagonized less completely. DADLE still decreased MAP significantly after injection of NMN. It also seemed to continue to decrease plasma noradrenaline after NMN and the lower dose of NML, although the decrease was no longer statistically significant (always in comparison with the corresponding group receiving saline infusion). Doses of NMN 1.3 mg kg^{-1} and NML 3 mg kg^{-1} were chosen for the following experiments in anaesthetized animals.

Experiments in anaesthetized rabbits

The plasma noradrenaline levels, MAP and heart rate of rabbits anaesthetized with pentobarbitone 60–80 mg kg^{-1}, as measured at $t = 49$ min, are also shown in Table 1. The control values resemble those obtained previously (Majewski *et al.*, 1983). As in pithed animals, NMN and NML produced transient hypotension when injected at $t = 45$ and $t = 40$ min, respectively. At $t = 49$ min, values of NMN-

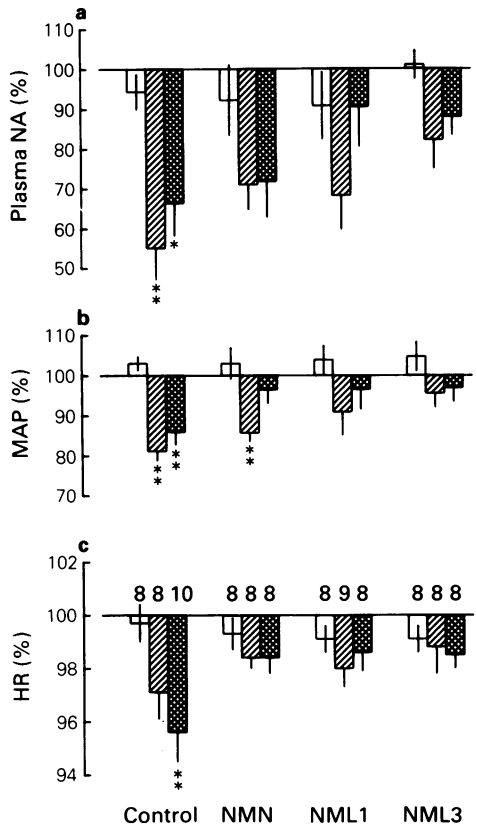

Figure 1 Interaction of opioid peptides and opioid antagonists on (a) plasma noradrenaline (NA) concentration, (b) mean arterial pressure (MAP) and (c) heart rate (HR) in pithed rabbits with electrically stimulated sympathetic outflow. Animals were either not pretreated (Control) or were pretreated with N-methyl naloxone 1.3 mg kg^{-1} (NMN) at $t = 45$ min or with N-methyl levallorphan (NML) 1 or 3 mg kg^{-1} at $t = 40$ min. Beginning at $t = 50$ min, physiological saline (open columns), [D-Ala^2-D-Leu^5]enkephalin 10 μg kg^{-1} min^{-1} (hatched columns) or dynorphin 1 μg kg^{-1} min^{-1} (cross-hatched columns) was infused for 5 min. Plasma NA, MAP and HR values at the end of the infusion are expressed as a percentage of the respective values before infusion. Each column represents the mean and vertical lines s.e.mean. The number of experiments is shown above each column in (c). Significant differences from respective saline-treated animals: $*P < 0.05$; $**P < 0.01$.

pretreated rabbits did not differ from controls; the plasma noradrenaline concentration and MAP of NML-pretreated animals, however, were significantly higher than in controls (Table 1).

Table 2 shows the effects of repeated infusion of DADLE 10 μg kg^{-1} min^{-1} and dynorphin 1 μg kg^{-1} min^{-1}. Both DADLE and dynorphin reduced the plasma noradrenaline concentration and MAP without a significant effect on heart rate. Whereas the effects of dynorphin remained approximately constant upon repeated infusion over 95 min, the effects of DADLE declined. In order to avoid such changes, the peptides were given only once for 5 min (beginning at $t = 50$ min) in all subsequent experiments.

Dose-response curves for DADLE and dynorphin are shown in Figure 2. DADLE 3, 10 and 30 μg kg^{-1} min^{-1} caused a dose-dependent fall in blood pressure, whereas the plasma noradrenaline level was decreased significantly only at 10 μg kg^{-1} min^{-1}, a dose that caused a slight increase in heart rate. Dynorphin 0.3, 1 and 3 μg kg^{-1} min^{-1} dose-dependently reduced both plasma noradrenaline and MAP. The highest dose also decreased heart rate.

The doses of 10 μg kg^{-1} min^{-1} DADLE and 1 μg kg^{-1} min^{-1} dynorphin were chosen to study the interaction with opioid antagonists (Figure 3). The effect of DADLE on the plasma noradrenaline level was not antagonized by NMN 1.3 mg kg^{-1} and also seemed to persist after NML 3 mg kg^{-1}. Moreover, its hypotensive effect was only slightly reduced by NMN and only reduced by about one half, but not abolished, by NML; the slight cardioacceleration also persisted, at least after NML. Both antagonists, in contrast, prevented the noradrenaline-decreasing effect of dynorphin. The hypotensive effect of dynorphin was greatly diminished by NMN 1.3 mg kg^{-1} and abolished by NML 3 mg kg^{-1}.

Discussion

The effects of DADLE and dynorphin in pithed rabbits, as observed in the present study, agree with those described previously (Szabo *et al.*, 1986). They are mediated by peripheral ganglionic receptors or (more probably) by presynaptic receptors at cardiovascular postganglionic sympathetic neurones; the receptor for DADLE is probably of the δ-type, the receptor for dynorphin of the κ-type (Szabo *et al.*, 1986). In initial experiments, doses of NMN (1.3 mg kg^{-1}) and NML (1 and 3 mg kg^{-1}) were determined that antagonized the peripheral effects of DADLE and dynorphin; DADLE was antagonized less completely than dynorphin. However, while our experiments show that NMN 1.3 mg kg^{-1} and NML 1 and 3 mg kg^{-1} are active peripherally, they do not allow us to conclude whether these doses are also selective for peripheral opioid receptors and devoid of central effects. Evidence for an exclusively peripheral action of NMN 1.3 mg kg^{-1} is the finding that

Table 2 Effect of repeated infusion of opioid peptides on plasma noradrenaline concentration, mean arterial pressure (MAP) and heart rate in anaesthetized rabbits

		P1	*P2*	*P3*	*P4*
Plasma noradrenaline (%)	Saline	99.0 ± 6.9	92.8 ± 5.7	105.1 ± 6.1	95.5 ± 8.1
	DADLE $10\,\mu g\,kg^{-1}\,min^{-1}$	73.2 ± 6.7*	86.2 ± 5.4	82.1 ± 4.3**	92.4 ± 9.0
	Dyn $1\,\mu g\,kg^{-1}\,min^{-1}$	68.6 ± 10.7*	72.2 ± 4.9*	75.3 ± 6.6**	64.0 ± 10.6*
MAP (%)	Saline	101.9 ± .0.5	100.6 ± 0.6	100.6 ± 0.7	100.5 ± 0.8
	DADLE $10\,\mu g\,kg^{-1}\,min^{-1}$	82.7 ± 3.2**	89.8 ± 0.6**	91.3 ± 1.1**	92.6 ± 1.2**
	Dyn $1\,\mu g\,kg^{-1}\,min^{-1}$	90.7 ± 1.2**	91.9 ± 1.0**	92.3 ± 0.9**	92.3 ± 1.1**
Heart rate (%)	Saline	99.2 ± 0.3	99.4 ± 0.2	99.6 ± 0.3	99.9 ± 0.2
	DADLE $10\,\mu g\,kg^{-1}\,min^{-1}$	99.9 ± 0.8	99.9 ± 0.6	99.5 ± 0.5	99.7 ± 0.5
	Dyn $1\,\mu g\,kg^{-1}\,min^{-1}$	99.4 ± 0.4	99.6 ± 0.4	100.4 ± 0.3*	99.8 ± 0.7

Physiological saline ($n = 17$), [D-Ala^2-D-Leu^5]enkephalin (DADLE; $n = 11$) or dynorphin (Dyn; $n = 10$) was infused four times for 5 min each. The infusion periods began at $t = 50$ (P1), $t = 80$ (P2), $t = 110$ (P3) and $t = 140$ min (P4). Plasma noradrenaline, MAP and heart rate at the end of the infusion periods are expressed as a percentage of the respective values before infusion. Means ± s.e.mean are given. Significant differences from respective saline-treated animals: $^*P < 0.05$, $^{**}P < 0.01$.

this dose did not antagonize the central depressant effect of [Met]enkephalin on renal sympathetic nerve activity in rabbits (Rhee *et al.*, 1985). Evidence for an exclusively peripheral action of NML 1 and $3\,mg\,kg^{-1}$ is the observation that even a dose of $75\,mg\,kg^{-1}$ exerted no central antagonist effect in mice (Bianchetti *et al.*, 1986). An entirely peripheral effect of NMN and NML will be assumed in the subsequent discussion of the experiments in anaesthetized rabbits.

As in pithed rabbits, DADLE and dynorphin caused a dose-dependent fall in blood pressure in anaesthetized rabbits. The fall was accompanied by a decrease in the plasma noradrenaline concentration (significant at only one dose of DADLE) and, at the highest dose of dynorphin, a decrease in heart rate. An apparent desensitization developed to the effects of DADLE but not to those of dynorphin. The observation resembles our previous finding of a selective desensitization of peripheral presynaptic δ- but not κ-receptor mechanisms in the rabbit isolated ear artery (Illes *et al.*, 1985) and in pithed rabbits (Ensinger *et al.*, 1986; Szabo *et al.*, 1986). The differential desensitization suggests that in anaesthetized rabbits, as in pithed rabbits and isolated tissues of the rabbit, DADLE and dynorphin acted through different receptors, probably δ- and κ-receptors, respectively.

In contrast to its lack of effect in pithed rabbits, NML increased MAP and the plasma noradrenaline level in anaesthetized rabbits (after a transient hypotension). It might be supposed that the increases were due to antagonism against an effect of endogenous opioids, released in the anaesthetized but not the pithed animals. Naloxone similarly increases blood pressure and the plasma noradrenaline concentration in conscious rabbits during hemorrhage (Schadt & Gaddis, 1985; Rutter *et al.*, 1987). However, NMN did not share the effects of NML in our experiments, and this casts some doubt on the involvement of opioid receptors.

What do the interaction experiments tell us about the location of the receptors mediating the effects of dynorphin and DADLE in anaesthetized rabbits? In the case of dynorphin, all effects were abolished or greatly attenuated by both NMN and NML, indicating an exclusively peripheral action. The results were less clear-cut in the case of DADLE. Its hypotensive effect was reduced by NMN and, to a greater extent, by NML, indicating a peripheral component. Yet, some hypotension persisted after the antagonists; moreover, the DADLE-induced decrease in the plasma noradrenaline level was not antagonized at all by NMN, and antagonism by NML was questionable. It seems unlikely that the NMN- and NML-resistant components were mediated through peripheral non-opioid receptor sites, since in pithed rabbits all effects of even higher doses of DADLE were abolished by naloxone (Szabo *et al.*, 1986). Rather, the antagonist-resistant components may have been central in origin and, hence, central as well as peripheral actions may contribute to the effects of DADLE. Central cardiovascular effects of the δ-selective peptide [Met]enkephalin, when given systemically, have been described previously (Eulie & Rhee, 1984; Rhee *et al.*, 1985).

The precise location of the peripheral sites of action of dynorphin and DADLE cannot be derived from the present experiments with certainty. A postsynaptic component in the hypotensive effect (see Introduction for references) seems unlikely: firstly, because in pithed rabbits DADLE and dynorphin failed to counteract the increase in blood pressure caused by infusion of noradrenaline (Szabo *et al.*,

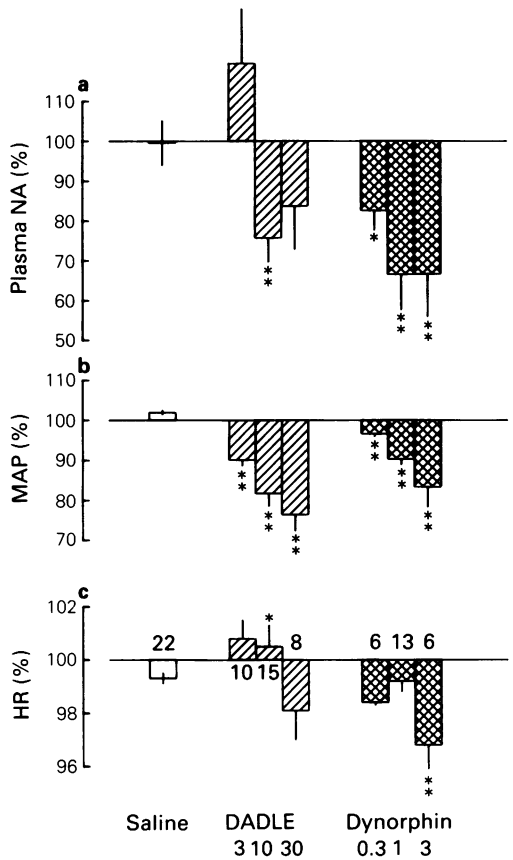

Figure 2 Dose-dependent effects of opioid peptides on (a) plasma noradrenaline (NA) concentration, (b) mean arterial pressure (MAP) and (c) heart rate (HR) in anaesthetized rabbits. Beginning at $t = 50$ min, physiological saline (open columns), [D-Ala^2-D-Leu^5]enkephalin (DADLE) (hatched columns) or dynorphin (cross-hatched columns) was infused for 5 min. Plasma NA, MAP and HR values at the end of the infusion are expressed as a percentage of the respective values before infusion. Each column represents the mean and vertical lines s.e.mean. The number of experiments is shown by each column in (c). DADLE 3, 10, and 30 $\mu g\,kg^{-1}\,min^{-1}$ and dynorphin 0.3, 1 and 3 $\mu g\,kg^{-1}\,min^{-1}$ were studied. Significant differences from saline-treated animals: $^*P < 0.05$; $^{**}P < 0.01$.

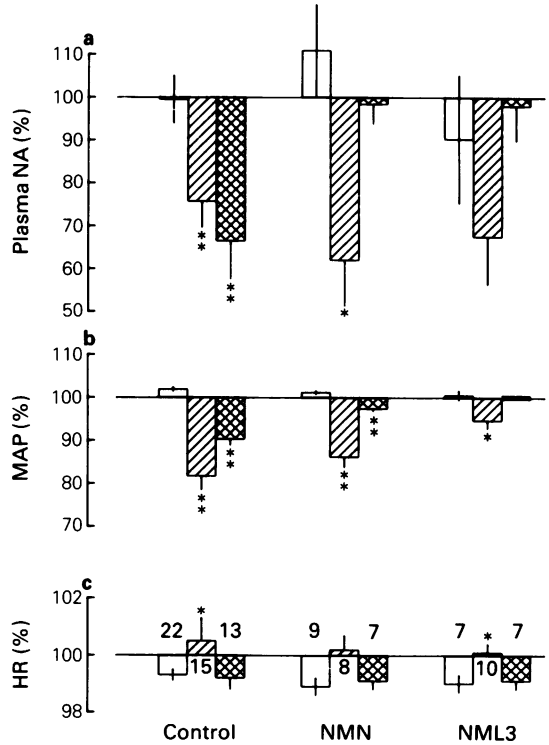

Figure 3 Interactions of opioid peptides and opioid antagonists on (a) plasma noradrenaline (NA) concentration, (b) mean arterial pressure (MAP) and (c) heart rate (HR) in anaesthetized rabbits. Animals were either not pretreated (Control) or were pretreated with N-methyl naloxone (NMN) 1.3 $mg\,kg^{-1}$ at $t = 45$ min or with N-methyl levallorphan (NML) 3 $mg\,kg^{-1}$ at $t = 40$ min. Beginning at $t = 50$ min, physiological saline (open columns), [D-Ala^2-D-Leu^5]enkephalin 10 $\mu g\,kg^{-1}\,min^{-1}$ (hatched columns) or dynorphin 1 $\mu g\,kg^{-1}\,min^{-1}$ (cross-hatched columns) was infused for 5 min. Plasma NA, MAP and HR values at the end of the infusion are expressed as a percentage of the respective values before infusion. Each column represents the mean and vertical lines s.e.mean. The number of experiments is shown by each column in (c). Significant differences from respective saline-treated animals: $^*P < 0.05$; $^{**}P < 0.01$.

1986), and secondly, of course, because the hypotension coincided with a decrease in noradrenaline release. The receptors might be located at pulmonary afferent fibres or in sympathetic ganglia (see Introduction). However, little is known about these receptors. The only peripheral opioid receptors that have been studied in detail are those located at terminal postganglionic sympathetic axons (see Introduction). The close similarity in the effects of the opioid peptides in anaesthetized rabbits, in pithed rabbits with electrically stimulated sympathetic outflow (effects via pulmonary afferents excluded), and in isolated cardiovascular tissues (pulmonary afferents and ganglionic effects excluded) suggests that, in the anaesthetized animals, the peripheral effects of dynorphin and DADLE are also mediated through presynaptic receptors in the sympathetic supply to the heart and blood vessels.

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