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The effect of enkephalins and of β-endorphin on the hypertensive response to physostigmine in the rat

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1 Intracarotid injection of [Leu]enkephalin and [Met]enkephalin produced a dose-dependent biphasic change in blood pressure of the rat consisting of an initial shortlasting fall followed by a longlasting increase of blood pressure. Naloxone consistently depressed or abolished the effects of enkephalins on blood pressure.

2 Intracarotid injection of β-endorphin only occasionally produced a hypotension, or did not produce any change in the blood pressure of the rat.

3 All three opioids ([Leu]enkephalin, [Met]enkephalin and β-endorphin) significantly depressed or abolished the hypertensive response to intravenous injection of physostigmine. This depressive action of opioids was easily reversed by naloxone.

5 It is concluded that opioids depress the central cholinergic link implicated in the hypertensive response to physostigmine most probably by inhibiting acetylcholine and/or noradrenaline release in the structures relevant for the action of physostigmine on blood pressure of the rat. This interaction is realized through the activation of opioid receptor(s).

Introduction

The intravenous injection of physostigmine is known to produce a hypertensive response in the rat anaesthetized with urethane. This effect is due to a central cholinergically mediated activation of peripheral sympathetic mechanisms (Varagić, 1955; Varagić, 1985; Brezenoff & Giuliano, 1982).

The endogenous opioid peptides have been shown to affect cardiovascular function via both central and peripheral mechanisms (Holaday, 1983). Enkephalins have been shown to modify the release of several neurotransmitter substances from the regions in the central nervous system. Inhibitory effects of enkephalins have been observed on the *in vitro* release of noradrenaline (Taube *et al.*, 1976), dopamine (Subramamian *et al.*, 1977) and of substance P (Jessel & Iversen, 1977). [Met]enkephalin and [Leu]enkephalin, as well as some of their derivatives, have also been shown to depress *in vivo* release of cortical acetylcholine following intraventricular injections in rats (Jhamandas *et al.*, 1977; Jhamandas & Sutak, 1980). Thus, it is possible that enkephalins have a general modulatory role on the release of several neurotransmitters. It was therefore interesting to study the possible interaction between opioid peptides and processes involved in the hypertensive response to physostigmine in the rat.

Methods

Wistar rats of either sex (200–250 g) were used in these experiments. The animals were anaesthetized with urethane (25% solution, 0.7 ml per 100 g subcutaneously).

The rats were prepared for direct recording of blood pressure from a carotid artery. This artery was connected via a polyethylene cannula to a small mercury manometer. The other carotid artery was also dissected and a T-cannula was inserted into it. The opioid peptides were injected into this carotid artery. The other drugs (physostigmine, naloxone, catecholamines) were injected into the jugular vein.

The following drugs were used: physostigmine salycylate, [Leu]enkephalin (Serva), [Met]enkephalin (Serva), β-endorphin (Sigma) naloxone (Du Pont), adrenaline hydrochloride and noradrenaline bitartrate. All drugs were dissolved in distilled water.

Results

The effects of [Leu]enkephalin, [Met]enkephalin and β-endorphin on blood pressure

The intracarotid injection of [Leu]enkephalin, in doses of 40, 100 and 200 $\mu g\,kg^{-1}$, produced either a pure

hypotension (6 experiments) or a biphasic response consisting of a short initial hypotension which was followed by a prolonged hypertension (5 experiments). The blood pressure response to intracarotid injection of [Leu]enkephalin was either depressed or completely blocked by naloxone (Figure 1). The observed changes in blood pressure after intracarotid injection of [Leu]enkephalin were not due to injection artefacts, because injection of the same volume of saline in the same manner, produced no changes in blood pressure.

The intracarotid injection of [Met]enkephalin, in doses of 40, 100 and 200 $\mu g\,kg^{-1}$, regularly produced a biphasic blood pressure response consisting of an initial shortlasting hypotension, followed by a prolonged hypertension (5 experiments).

The blood pressure responses to intracarotid injections of [Met]enkephalin were regularly blocked by naloxone (0.2 $mg\,kg^{-1}$, intravenously).

The increasing doses of naloxone itself injected intravenously (50, 200 and 500 $\mu g\,kg^{-1}$) produced no change in the blood pressure. The intracarotid injection of naloxone (0.2 $mg\,kg^{-1}$) also produced no change in the blood pressure (Figure 2).

The intracarotid injection of β-endorphin (50 $\mu g\,kg^{-1}$) occasionally produced a slight to moderate hypotension, or produced no change in blood pressure.

The intracarotid injection of enkephalins also produced a biphasic response in heart rate. The initial hypotensive response to intracarotid injections of both [Leu] and [Met]enkephalin (40 and 100 $\mu g\,kg^{-1}$) was coupled with mild bradycardia, whereas the hypertensive effect of enkephalins was associated with slight tachycardia. However, these changes were not significant.

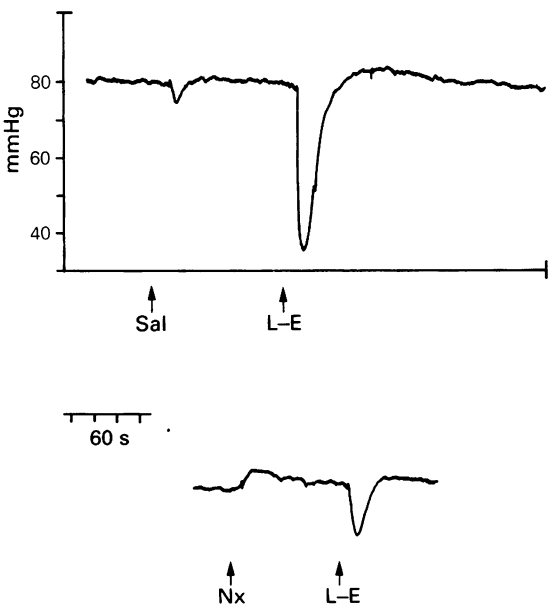

Figure 1 The antagonistic effect of naloxone (Nx, 0.2 $mg\,kg^{-1}$, intravenously) on the blood pressure response to [Leu]enkephalin (L-E, 40 $\mu g\,kg^{-1}$, intra-carotid). Sal: intracarotid injection of 0.1 ml saline. Time: 1 min intervals.

The effect of [Leu]enkephalin on the hypertensive response to physostigmine

The intracarotid injection of [Leu]enkephalin in doses of 40 and 100 $\mu g\,kg^{-1}$ consistently and significantly depressed the hypertensive response to physostigmine (50 $\mu g\,kg^{-1}$ intravenously). In all five experiments from this series physostigmine produced an increase of blood pressure of 24 ± 2 mmHg. After intracarotid injection of [Leu]enkephalin (40 $\mu g\,kg^{-1}$), the same dose of physostigmine produced significantly less hypertension (Table 1). The duration of the hypertensive response to physostigmine was also decreased by [Leu]enkephalin. The intravenous injection of naloxone (0.2 $mg\,kg^{-1}$) significantly antagonized the depressive action of [Leu]enkephalin on the physostigmine hypertension. The antagonistic effect of naloxone was more prominent on the amplitude of blood pressure than on the duration of the hypertensive response (Table 1).

The effect of [Met]enkephalin on the hypertensive response to physostigmine

The intracarotid injection of [Met]enkephalin in doses of 40, 100 and 200 $\mu g\,kg^{-1}$ also consistently and significantly depressed the hypertensive response to physostigmine (50 $\mu g\,kg^{-1}$, intravenously). Both amplitude and duration of the hypertensive effect of physostigmine were depressed. Here again, naloxone (0.2 $mg\,kg^{-1}$) antagonized the effect of [Met]enkephalin, and the antagonistic effect was more prominent on the amplitude than on the duration of the hypertensive response to physostigmine.

It is of interest that a depressive effect of [Met]enkephalin on the physostigmine-induced hypertension was observed only after the first dose of [Met]enkephalin (40 $\mu g\,kg^{-1}$). Higher doses produced no depression because an acute tolerance developed towards the blood pressure effect of [Met]enkephalin (Table 1).

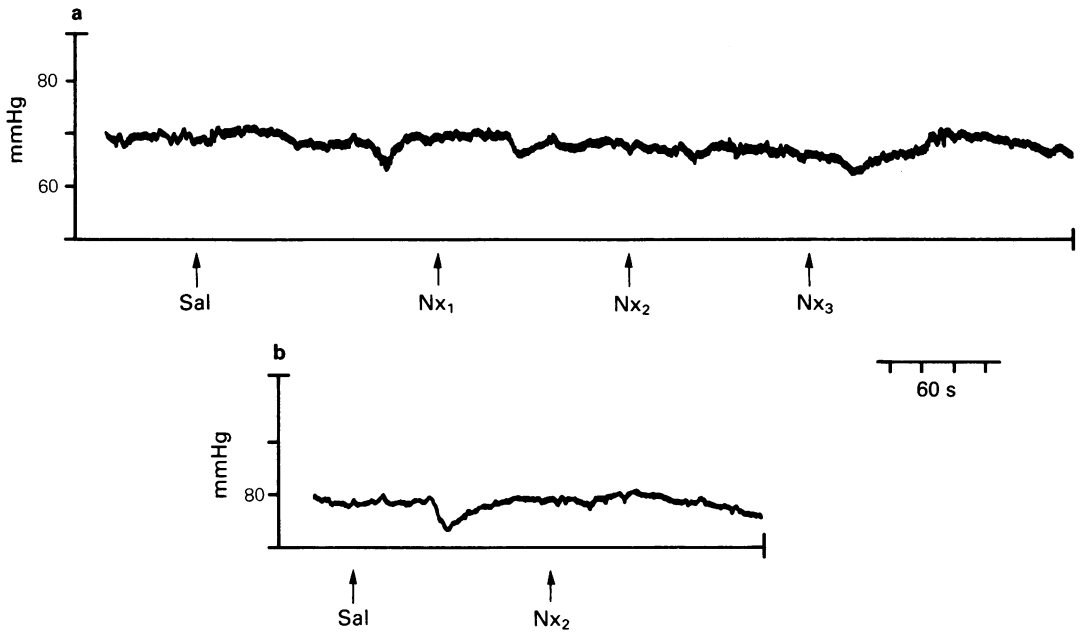

Figure 2 The effect of increasing doses of naloxone on the blood pressure of the rat. (a) Effects after intravenous injections of 50 $\mu g\,kg^{-1}$ (Nx_1), 200 $\mu g\,kg^{-1}$ (Nx_2) and 500 $\mu g\,kg^{-1}$ (Nx_3). Sal: injection of the corresponding volume of saline. (b) Blood pressure response to intracarotid injection of 200 $\mu g\,kg^{-1}$ (Nx_2) naloxone. Time: 1 min intervals.

The effect of β-endorphin on the hypertensive response to physostigmine

The intracarotid injection of β-endorphin in a dose of 50 $\mu g\,kg^{-1}$ produced either a significant depression, or even a complete block of the hypertensive response to physostigmine (50 $\mu g\,kg^{-1}$ intravaneously). Both the amplitude and duration of the hypertensive response to physostigmine were depressed. Intravenous injection of naloxone (0.2 $mg\,kg^{-1}$) effectively antagonized the inhibitory action of β-endorphin on the hypertensive response to physostigmine, the effect on

Table 1 The effects of intracarotid injections of [Leu]enkephalin, [Met]enkephalin and β-endorphin on the hypertensive blood pressure response to intravenous injection of physostigmine

		Effect of physostigmine		
	Treatment	(a) *Hypertension* (mmHg)	(b) *Duration* (min)	P
1	Physostigmine (Ph, 50 $\mu g\,kg^{-1}$, i.v.)	105 ± 5(6)	23 ± 2(6)	(1a:1b) < 0.05
2	[Leu]enkephalin (40 $\mu g\,kg^{-1}$, i.c.) + Ph	94 ± 6(5)	9 ± 1.5(5)	(1a:3a) < 0.01
3	[Leu]enkephalin (100 $\mu g\,kg^{-1}$, i.c.) + Ph	90 ± 7(5)	6 ± 1.5(5)	(1b:2b) < 0.001
4	Naloxone (0.2 $mg\,kg^{-1}$, i.v.) + Ph	106 ± 6(5)	12 ± 2(5)	(1b:3b) < 0.001
5	[Met]enkephalin (40 $\mu g\,kg^{-1}$, i.c.) + Ph	96 ± 9(6)	12 ± 2(11)	(1a:5a) < 0.02
6	[Met]enkephalin (100 $\mu g\,kg^{-1}$, i.c.) + Ph	104 ± 4(6)	12 ± 3(5)	(1b:5b) < 0.001
7	[Leu]enkephalin (200 $\mu g\,kg^{-1}$, i.c.) + Ph	107 ± 5(6)	13 ± 2(5)	(1a:6a) not sign (1a:7a) not sign
8	β-Endorphin (50 $\mu g\,kg^{-1}$, i.c.) + Ph	88 ± 5(5)	9 ± 2(5)	(1a:8a) < 0.05
9	Naloxone (0.2 $mg\,kg^{-1}$, i.v.) + Ph	103 ± 6(5)	12 ± 2(5)	(1b:8b) < 0.001

The values represent absolute readings of blood pressure in mmHg (mean ± s.e.mean.). The number of experiments is given in parentheses.

amplitude being more pronounced than on the duration of the response. A typical experiment is shown in Figure 3. All the experiments from this series are statistically evaluated in Table 1.

The effect of [Leu]enkephalin on the blood pressure response to catecholamines

The increasing doses of [Leu]enkephalin (50, 100 and 200 $\mu g\,kg^{-1}$), injected into the carotid artery, did not significantly alter the blood pressure response to adrenaline and noradrenaline (Table 2).

Discussion

The enkephalins and β-endorphin undergo rapid destruction in the peripheral circulation due to the action of various peptidases. On the other hand, there is little, if any, evidence in support of specific blood-brain barrier transport for peptides, including opioids (Pardridge & Meitus, 1981). However, these data do not rule out the possibility of transport if sufficiently high concentrations are used. To facilitate transport to the brain, in our series of experiments the opioids were injected into the carotid artery. Thus, breakdown of opioids in the peripheral circulation was largely avoided and a high concentration of peptides was achieved at the level of brain capillary endothelial wall, i.e. the blood-brain barrier.

The pressor response to physostigmine is presumed to involve a central cholinergic link, with an activation of central cholinergic mechanisms, which subsequently trigger the peripheral sympathetic activation (Varagić & Krstić, 1966; Varagić *et al.*, 1969). Two possible sites of cholinergically-mediated pressor response to physostigmine are nucleus tractus solitarius and the locus coeruleus (Brezenoff & Giuliano, 1982). On the other hand, the fact that naloxone antagonizes hypotension and increases the survival in various shock states, indicates a function of the endogenous opioids in cardiovascular regulation (Holaday, 1983). It is therefore possible that opioids interact in some way with a central cholinergic link involved in the blood pressure rise produced by physostigmine.

The nature of the modulating action of opioids in

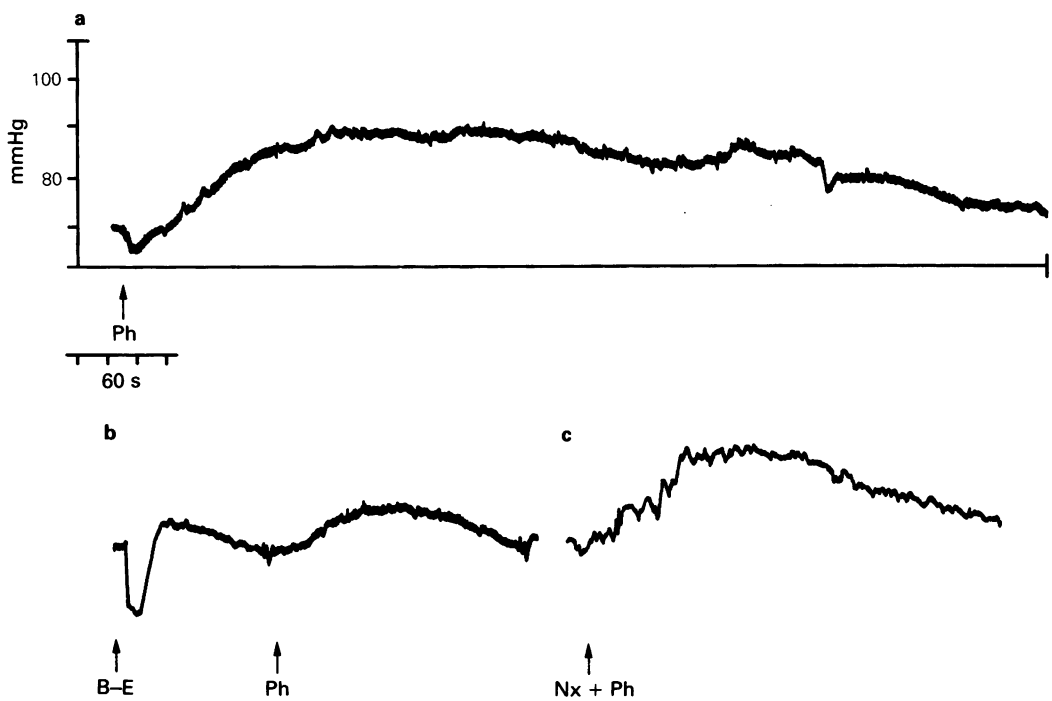

Figure 3 The effect of β-endorphin on the blood pressure response to physostigmine (Ph, 50 $\mu g\,kg^{-1}$, intravenously). (a) Control response to physostigmine. At B-E, β-endorphin 50 $\mu g\,kg^{-1}$ injected into the carotid artery; (b) shows the response to physostigmine, 5 min after injection of β-endorphin; (c) shows the response to physostigmine after previous intravenous injection of naloxone (Nx, 0.2 $mg\,kg^{-1}$).

Table 2 Influence of increasing doses of [Leu]enkephalin on the blood pressure response to catecholamines in the rat

Blood pressure rise as % increase over control, prior to administration of drugs							
		After [Leu]*enkephalin*					
Control	*50 μg kg⁻¹, i.c.*		*100 μg kg⁻¹, i.c.*		*200 μg kg⁻¹, i.c.*		
NA	Ad	NA	Ad	NA	Ad	NA	Ad
40.9 ± 6.3 (4)	50.7 ± 4.7 (4)	41.6 ± 4.7 (4)	50.3 ± 6.8 (4)	47.9 ± 4.4 (4)	56.9 ± 5.7 (4)	40.2 ± 4.9 (4)	52.5 ± 7.5 (4)

Na = noradrenaline (5 μg kg^{-1}, i.v.); Ad = adrenaline (2.5 μg kg^{-1}, i.v.); i.c. = 1 carotid artery.

the central nervous system is still controversial. Enkephalins have been shown to modify the release of several neurotransmitter substances from several regions in the central nervous system. The enkephalins have been shown to inhibit *in vitro* release of noradrenaline (Taube *et al.*, 1976), substance P (Jessel & Iversen, 1977) and of dopamine (Subramanian *et al.*, 1977). Both [Met]- and [Leu]enkephalin have been shown to depress *in vitro* release of cortical acetylcholine, following intraventricular injection of these substances in rats (Jhamandas *et al.*, 1977; Cox & Goldstein, 1978). Morphine and related opiates have been known for a long time to inhibit acetylcholine release at the peripheral cholinergic synapses (Paton, 1957). Therefore, enkephalins have been postulated to have a general modulating action on the release of several neurotransmitters in the central nervous system. This action is realized through opioid receptors. Our experiments are in agreement with prevous findings, because naloxone consistently reversed the antagonistic action of opioids on the blood pressure rise produced by physostigmine.

This assumption is further supported by our finding that the blood pressure response to exogenous catecholamines remains unaffected by opioids, thus indicating that the peripheral adrenoceptors are not influenced by opioids, when given by intracarotid injection.

Various opioid peptides, after injection into the carotid artery, in the present experiments produced different responses. The increasing doses of [Leu]enkephalin produced a pure hypotension in about 50% of experiments, or a biphasic response with shortlasting initial hypotension followed by a secondary prolonged hypertension in another 50% of experiments. Met-enkephalin regularly produced a biphasic blood pressure response consisting of hypo- and hypertension. Beta-endorphin by itself produced only occasionally a hypotensive effect, or did not produce any change in blood pressure. These effects of opioid peptides are presumably due to activation of the opioid receptors because they can be blocked by naloxone. This substance is known to be more potent in antagonizing the effects of μ-receptor agonists than κ- or σ-receptor agonists and is thought to have highest affinity for the μ-receptors (Martin, 1983; Snyder, 1984). Thus, it can be speculated that the blood pressure response to opioids under the conditions of the present experiments might be preferentially due to μ-receptor activation, although other opioid receptors cannot be excluded. The dose of naloxone in our experiments was sufficiently high that the overlapping of various opioid receptors is possible.

It can be concluded that [Leu]enkephalin and [Met]enkephalin, as well as β-endorphin, depress the central cholinergic processes implicated in the hypertensive blood pressure response to physostigmine most probably by inhibiting acetylcholine and/or noradrenaline release from the corresponding neurones. This interaction is probably realized through the activation of opioid receptor(s), because it can be blocked by naloxone.

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