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Sodium deprivation increases the antinociceptive activity of morphine

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In rats maintained for 50 days on a low-sodium diet and with a compensatory hyperactivity of the renin-angiotensin system, the antinociceptive activity of morphine was significantly longer-lasting than in controls. It is suggested that the renin-angiotensin system modulates opioid system responsivity.

Various correlations seem to exist between CNS mechanisms responsible for pain sensitivity and those responsible for blood pressure and regulation of hydration. The brain contains an endogenous renin-angiotensin system independent of renal origin (Ganten et al 1983); angiotensin II (A) has been immunohistochemically localized in neuronal cell bodies and terminals in the CNS (Fuxe et al 1976; Changaris et al 1978); specific receptors and enzymes necessary for A formation and degradation are present in defined brain regions (subfornical organ, organ vasculosum lamina terminalis, midbrain, preoptic-septal areas, magnocellular nuclei of the hypothalamus) (Bennett & Snyder 1976; Sirett et al 1977; Buggy et al 1978).

On the other hand, endogenous opioid peptides are present in CNS regions known to regulate blood pressure and hydration, and to contain A terminals, cell bodies and/or receptors (Fuxe et al 1976; Sar et al 1978). In addition, enzymes which are involved in the biosynthesis of angiotensin I and II also metabolize opioid peptides (Benuck & Marks 1978; Erdos 1979).

Intraliquoral injection of morphine and β-endorphin produces hypotension (Feldberg & Wei 1978), while naloxone has been reported to reverse the hypotensive effect of clonidine and α-methyldopa in spontaneously hypertensive rats (Farsang et al 1980), and clonidine and α-methylnoradrenaline induce the release of a β-endorphin-like peptide from brain stem slices of these rats (Kunos et al 1981). Antiserum to β-endorphin causes a rise in pressure on injection into the nucleus tractus solitarii in rats (Petty et al 1982). Moreover naloxone is effective both in animals and in man in counteracting shock induced by hypovolaemia, endotoxins or spinal cord injury (Faden & Holaday 1979; Zamir & Segal 1979; Holaday & Faden 1980). Conversely, an altered pain sensitivity has been demonstrated in genetically and experimentally hypertensive rats as measured by the hot plate method (Zamir et al 1980), and in hypertensive humans (Zamir & Shuber 1980).

On the whole, these accumulating data prompted us to study the antinociceptive activity of morphine in a state of amplification of the renin-angiotensin system in rats.

Materials and methods

A total of 72 experimental and 63 control rats of a Wistar strain (Morini, S. Polo d'Enza, Reggio nell'Emilia, Italy), 240–250 g, were used. Experimental rats had free access for 50 days to a low-sodium diet prepared according to Orent-Keiles et al (1937): the sodium content of this diet was 0·01%. Control rats were maintained on a standard diet with a sodium content of 0·4%.

Basal pain threshold and antinociceptive activity of morphine (morphine sulphate, Carlo Erba, Milan, 5 mg kg^{-1} i.p.) were determined by the hot-plate test, with a platform temperature of 55·5 ± 0·1 °C, and a cut-off time of 60 s, by two of the authors, who were blind to the treatment. The effect of morphine was calculated as a percentage of the maximum possible effect (MPE) according to the formula: $(TL - BL)/(60 - BL) \times 100$ (TL = test latency, in seconds; BL = baseline latency, in seconds; 60 = cut-off time of 60 seconds) (Harris & Pierson 1964). Eight sodium-deprived and 8 control rats were decapitated at the end of the experiment, and blood was collected. The sodium concentration in serum was determined by flame photometry (Zeiss PMQ II). Plasma renin activity (PRA) was measured as follows: plasma was thawed, buffered to pH 5·5 with citric acid (0·38 M) and incubated for 60 min at 37 °C (controls at 4 °C) in the presence of 8-hydroxyquinoline and dimercaprol. After incubation, aliquots of plasma were radioimmunoassayed for angiotensin I using a Renin Assay kit (New England Nuclear). PRA is expressed as ng of angiotensin I generated per millilitre of plasma per hour of incubation. Student's *t*-test was used for statistical analysis of data, $P < 0.05$ being accepted as a significant difference. Variability is expressed as the standard error of the mean.

Results and discussion

The baseline reaction latency on the hot plate in rats fed a low-sodium diet was approximately the same as in rats

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Table 1. Influence of sodium deprivation on pain threshold and morphine activity, in rats. Hot plate test (55·5 °C).

Group*	Baseline reaction latency (time s ± s.e.) (immediately before treatment)	Treatment i.p.	% Increase in reaction latency (MPE)** at the following times (min) after treatment 15	30	60	90	120
1: Standard diet (30)	5·28 ± 0·28	Saline	−3·72 ± 1·47	1·92 ± 1·79	−1·39 ± 1·73	−1·95 ± 1·51	−2·24 ± 1·66
2: Standard diet (33)	5·90 ± 0·37	Morphine, 5 mg kg^{-1}	19·38 ± 5·34	10·11 ± 3·20	2·58 ± 0·99	2·46 ± 2·20	1·82 ± 1·40
3: Low-sodium diet for 50 days (42)	5·59 ± 0·30	Morphine, 5 mg kg^{-1}	30·03 ± 5·44	44·58 ± 5·58†	37·49 ± 6·24†	20·71 ± 10·20	6·77 ± 6·42
4: Low-sodium diet for 50 days (30)	5·45 ± 0·20	Saline	1·47 ± 1·37	1·42 ± 1·27	−4·21 ± 1·31	−3·48 ± 1·19	−2·38 ± 1·01

* No of rats in parentheses. ** See Methods for details. †$P < 0·001$ (compared with group 2) (Student's t-test).

fed a standard sodium diet; on the other hand, the antinociceptive effect of morphine was much longer-lasting in sodium-deprived rats than in controls (Table 1). The involvement of sodium in the action of narcotic analgesics is well established: in-vitro, sodium increases antagonist binding while reducing agonist binding to the opiate receptor (Pert & Snyder 1974; Simon et al 1975); in-vivo, large quantities of NaCl, given intraperitoneally to mice, decrease the antinociceptive activity of morphine (Lujan et al 1978), while hydrochlorothiazide, causing an acute sodium loss, has an analgesic effect and potentiates the analgesic activity of morphine (Poggioli et al 1985). However, in our experimental conditions, the sodium concentration in serum, measured at the end of the experiment, was not significantly different in the two groups of rats (371·25 ± 8·67 and 380·60 ± 12·24 mg/100 ml in sodium-deprived and in control rats, respectively): rats maintained on a low-sodium diet have a compensatory hyperactivity of the renin-angiotensin system (Vollmer et al 1981) and return to sodium balance within a short time (Sweet et al 1981). In our experimental conditions PRA was 7·2 ± 1·1 (ng of angiotensin 1 ml^{-1} h^{-1}) in sodium-deprived rats, and 2·1 ± 0·6 in controls ($P < 0·05$). So, it seems most likely that a positive correlation exists between the renin-angiotensin system and the antinociceptive activity of morphine.

A greater antinociceptive response to morphine has been observed in spontaneously hypertensive rats than in normotensive rats (Bhargava 1982), and a difference in brain opiate receptors sensitivity has been suggested to explain this finding. Our results show that the antinociceptive effect of morphine is much longer-lasting in sodium-deprived rats than in controls: this could be the consequence either of morphine being more strongly bound to its receptors or of its delayed metabolism and elimination. Endogenous opioid peptides (leucine-enkephalin, methionine-enkephalin, and β-endorphin), as well as morphine, inhibit the central actions of angiotensin (Summy-Long et al 1981), and it has been suggested that the endogenous opioid system may modulate the central angiotensin system (Summy-Long et al 1981). The present results, showing that in a state of amplification of the renin-angiotensin system the antinociceptive effect of morphine is greatly increased and prolonged, seem to indicate that a reciprocal modulation might be operative between these two peptidergic systems.

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Effects of morphine and clonidine on sulphobromophthalein disposition in mice

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Levels of sulphobromophthalein (BSP) in plasma and liver were elevated by the opiate, morphine, and by the α_2-adrenoceptor agonist, clonidine. Neither morphine, 1 mg kg^{-1}, nor clonidine, 0·01 mg kg^{-1}, affected BSP levels significantly. When given together at these doses, they caused BSP levels in plasma and liver to be raised. At 20 mg kg^{-1}, the effect of morphine on BSP levels was maximal, as was that of clonidine, 1·0 mg kg^{-1}. However, the effect of these drugs given together on plasma BSP exceeded the maximal effect of either alone. Yohimbine, an α_2-adrenoceptor antagonist, did not affect BSP levels, nor did the opiate antagonist, naloxone. Each of these antagonists reversed the hepatobiliary effects of its respective agonist, as shown by return of BSP levels to those of saline-treated mice. Yohimbine did not reverse morphine, nor did naloxone reverse clonidine. The additive effects of morphine and clonidine and the specificities of their respective antagonists strongly suggest the involvement of discrete receptors mediating their essentially identical hepatobiliary effects.

Several studies in the past decade have shown interactions between opiates and the adrenergic nervous system. Schultzberger et al (1978) and Wilson et al (1980) showed that opioid peptides are found in adrenergic nerve endings and in the adrenal medulla, which are both sites of catecholamine storage. In the locus coeruleus, both opiate and adrenergic nerve endings have been identified (Aghajanian 1982). Clonidine, an α_2-adrenergic agonist, has been shown to alleviate the clinical symptoms of opiate withdrawal (Gold et al 1978; Washton et al 1980). Furthermore, morphine has an effect on adrenergic transmission (Hughes et al 1975) and noradrenaline release in-vitro (Montel et al 1974).

Sulphobromophthalein (BSP) is an anionic dye which has been used extensively to evaluate hepatobiliary function. Upon intravenous administration, this dye is rapidly taken up by the liver, conjugated with glutathione and secreted into bile. Prolonged retention of BSP in plasma is regarded as evidence of hepatobiliary dysfunction, reflecting impairment of hepatic blood flow, parenchymal cell functions or biliary secretion and flow (Kaplowitz et al 1982). Although currently BSP disposition is infrequently studied in clinical settings, it is still widely used to investigate hepatobiliary function. We have recently shown that opiates and the α_2-adrenergic agonist, clonidine, have nearly identical effects on the hepatobiliary disposition of BSP (Hurwitz et al 1985; Ben-Zvi & Hurwitz 1985). Both elevate BSP levels in liver and plasma, while reducing amounts excreted in bile. We, therefore, chose to study interactions between clonidine and morphine and their specific antagonists on the retention of BSP in plasma and liver.

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Methods

Male Swiss Webster mice, 25–35 g were housed, 10 per cage, over crushed corn cob bedding at 23–25 °C. A 12 h dark–light cycle was followed, which was switched at 0600 and 1800 h. Purina rodent chow and water were freely available. Mice were originally obtained from Arther Sutter Co., Springfield, MO, and subsequently from Lab Supply Co., Indianapolis, IN. In each experiment, we report results from animals that had been delivered from a single supplier. Sulphobromophthalein sodium (BSP) was obtained from Hynson Westcott and Dunning, Baltimore, MD, and from Aldrich Chemical Co., Milwaukee, WI. Morphine sulphate was obtained from Eli Lilly, Indianapolis, IN; clonidine hydrochloride was from Boehringer Ingelheim, Ridgefield, CT; naloxone hydrochloride was from Endo Laboratories, Garden City, NY; and yohimbine hydrochloride was from Sigma Chemical Co., St Louis, MO, USA.

In a typical experiment, mice were injected subcutaneously with saline, morphine or clonidine or their antagonists 30 min before intravenous BSP. Thirty minutes after dye administration, blood was obtained from the orbital sinus and the animals were killed and their livers removed for dye determination. At these times, morphine and clonidine had their maximal effects on BSP disposition (Hurwitz et al 1985; Ben-Zvi & Hurwitz 1985). BSP was analysed in plasma spectrophotometrically at 580 nm after appropriate dilution of samples in 0·1 M sodium hydroxide. The dye content of