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RELEASE OF VASOPRESSIN BY ENKEPHALIN

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Leu-enkephalin, its stable analogue [D-Ala^2-D-Leu^5]-enkephalin and the C-fragment of lipotropin (β endorphin) injected intravenously in the rat produced antidiuretic responses which were inhibited reversibly by naloxone. It was shown for Leu-enkephalin that injection into the cerebral ventricles was at least ten times more effective than intravenous injection and for [D-Ala^2-D-Leu^5]-enkephalin that the antidiuretic response was associated with increased excretion of vasopressin in the urine.

Introduction The extent to which enkephalin reproduces the actions of morphine on the central nervous system continues to excite interest. Morphine has an antidiuretic action, first observed by de Bodo (1944), which has been attributed to the release of vasopressin from the neurohypophysis. In the dog, direct stimulation of neurosecretory cells has been demonstrated by injection of morphine into the supraoptic nucleus of the anterior hypothalamus (Duke, Pickford & Watt, 1951). However, intravenous injections of morphine may cause a fall in blood pressure which could induce reflex release of vasopressin (Bisset, Black, Hilton, Jones, Kanjanapothi & Montgomery, 1974). Recently, it has been shown in the rat that morphine can produce without hypotension an antidiuretic action which is blocked by an antibody to vasopressin and accompanied by increased excretion of vasopressin in the urine (Kanjanapothi, 1975). In this paper enkephalin and C-fragment of lipotropin (β endorphin) are shown to produce a similar antidiuretic action.

Methods Water-loaded rats under ethanol anaesthesia, in which a constant fluid load equivalent to 8% body weight was maintained (Clarke & Rocha e Silva, 1967), were used for obtaining antidiuretic responses, collecting urine samples and assaying extracts of urine for antidiuretic activity (ADA). Carotid arterial blood pressure was usually measured simultaneously with urine flow. Injections were made intravenously (i.v.) into an external jugular vein or intraventricularly (i.c.v.), in a volume of 10–25 µl, through a cannula previously inserted into a lateral cerebral ventricle. Control i.c.v. injections of 25 µl 0.9% w/v NaCl solution (saline) had no effect on urine flow. Vasopressin was extracted from 5 ml samples of urine by adsorption on Spherosil glass beads and elution with acetone (Ratcliffe & Edwards, 1971). Extracts were assayed for ADA against vasopressin (Pitressin, Parke-Davis). Results have been corrected for a measured loss of hormone (57%) on extraction. Leu-enkephalin was synthesized by Dr J. F. Collins, Department of Chemistry, Sir John Cass Institute of Science, London, and [D-Ala^2-D-Leu^5]-enkephalin by Dr S. Wilkinson, Wellcome Research Laboratories, Beckenham, Kent. C-fragment extracted from porcine pituitary glands was obtained from Dr D. G. Smyth, National Institute for Medical Research, London. We are grateful for gifts of these materials. Doses of morphine are expressed as the sulphate.

Results Figure 1 illustrates the antidiuretic action if i.v. Leu-enkephalin in the rat. A dose of 10 µg produced an antidiuretic response which was delayed in onset until the third minute after injection and lasted for 30 minutes. The maximum decrease in urine flow in any one min was 27%. In contrast, 50 µu vasopressin (not shown) produced a response with an earlier onset starting in the second minute, a greater maximum fall of 55% and a shorter duration of 15 minutes. The profiles of the antidiuretic responses to 40 µg Leu-enkephalin and 200 µu (approximately 500 pg) vasopressin were similar initially but the recovery was slower with Leu-enkephalin. Naloxone (10 µg i.v.) which itself had no antidiuretic action inhibited almost completely the response to 40 µg Leu-enkephalin; recovery from the inhibition occurred within 30 minutes. Similar results were obtained in five other rats: the lowest threshold dose of Leu-enkephalin was 5 µg. The i.v. injections of Leu-enkephalin caused a rise in blood pressure of 5–10 mmHg usually lasting only 5 minutes. In one experiment the antidiuretic response to 40 µg Leu-enkephalin i.v. was matched by that to only 2.5 µg morphine i.v. but this produced a fall in blood pressure of 25 mmHg which might have accounted at least in part for the antidiuresis.

C-fragment injected i.v. in doses of 10–40 µg produced antidiuretic responses very similar to those shown in Figure 1 for the same doses of Leu-enkephalin.

An antidiuretic response was produced with both Leu-enkephalin and morphine injected i.c.v. and in doses at least ten times smaller than those required in the same rat for a similar response by the i.v. route.

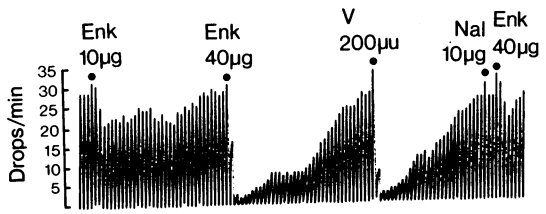

Figure 1 Urine flow in the anaesthetized rat measured with a drop recorder which reset to zero every min (1 drop = 4.3 μl). Each injection was made i.v. at the beginning of the minute marked with a dot. Enk = Leu-enkephalin; V = vasopressin; Nal = naloxone.

In an experiment with [D-Ala^2-D-Leu^5]-enkephalin, three successive 5 ml samples of urine were collected. At the beginning of the second collection, [D-Ala^2-D-Leu^5]-enkephalin 200 μg was injected i.v. This produced an antidiuretic response much more prolonged than that observed with similar doses of Leu-enkephalin. There was a brief initial rise in blood pressure. The times required for excretion of the three 5 ml samples were, respectively: 28, 108 and 44 minutes. The samples of urine were extracted and the extracts assayed for ADA. The total amounts of ADA excreted were <21, 253 and 44 μu and the rates of excretion <0.75, 2.34 and 0.62 μu/minute. The ADA of the extracts was abolished by incubation with 0.01 M sodium thioglycollate (Bisset, 1961) but persisted after the response to [D-Ala^2-D-Leu^5]-enkephalin had been blocked by naloxone.

Discussion The reversible inhibition of the antidiuretic responses to enkephalin and C-fragment by naloxone suggests that the receptors involved are similar to those for morphine. The increased rate of excretion of ADA, identified with vasopressin by the thioglycollate test, during the antidiuretic response to [D-Ala^2-D-Leu^5]-enkephalin, provides evidence that this response is mediated by release of vasopressin from the neurohypophysis. Approximately 13% of a dose of vasopressin injected i.v. in the rat is excreted in a biologically active form in the urine (Kanjanapothi, 1975). Therefore, the excretion of 253 μu ADA in the first 5 ml sample of urine collected after injection of [D-Ala^2-D-Leu^5]-enkephalin would represent a release of about 1.9 mu vasopressin into the circulation. The absence of a hypotensive response to enkephalin, the delay in onset of the antidiuretic response observed with small doses and the greater effectiveness by the i.c.v. than the i.v. route, indicate a central site of action. This might involve direct stimulation of neurones in the supraoptic or paraventricular nuclei of the anterior hypothalamus, or, as demonstrated recently with nicotine in the cat, (Bisset, Feldberg, Guerzenstein & Rocha e Silva, 1975), excitation of an afferent neural pathway to those nuclei.

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