Behavioural effects of naloxone in rats

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Summary. Naloxone in rats induces a behavioural syndrome closely resembling that induced by intraliquorally injected ACTH peptides. This effect is probably due to a displacement of the ACTH peptides from other receptors (e.g. opiate receptors).

Naloxone is considered to be a pure narcotic antagonist devoid of any other pharmacological activity in itself, although a few reports mention a potentiation of some effects of apomorphine[2-4]; in other words, the sole effect of naloxone seems to consist in the binding of the opiate receptors, which are blocked and cannot be activated by their agonists.

It has been found that some ACTH fragments, in addition to enkephalins, endorphins and other peptides, also have binding affinity for the opiate receptors in the brain[5-8]. It is known that ACTH peptides (ACTH, β^{1-24}-ACTH, α- and β-MSH, β-LPH, CRF), intraliquorally injected, induce a well-defined behavioural syndrome[9-12] characterized by a great increase in the number of stretchings, yawnings and spontaneous penile erections; there is also EEG arousal and, in female rabbits, sexual excitement (lordosis and tail deviation), LH release and ovulation. Furthermore, ACTH peptides exert a profound influence on the extinction behaviour of rats[13-17].

The above mentioned extra-hormonal effects of ACTH and related peptides lead one to suppose that, in the brain, they have affinity for various receptors which, in turn, are very versatile and can bind various molecules.

Accordingly we set out to ascertain whether naloxone might reciprocally bind (and possibly activate) not only the opiate receptors but also those responsible for the behavioural syndrome produced by ACTH.

Material and methods. Adult male Wistar rats, weighing 280-330 g, were used. Naloxone hydrochloride was s.c. injected at doses of 1,5 or 10 mg/kg; β^{1-24}-ACTH (Synacthen CIBA) was injected into a lateral brain ventricle without anaesthesia at doses of 0.5 or 1 µg/rat in a constant volume of 10 µl, 30 min after the s.c. injection of naloxone; the control animals were treated with saline, s.c. and intraventricularly. Immediately after treatment the rats were placed in glass cages and carefully observed for 2 h. Penile erections, yawnings and stretchings were scored. Penile erections arise suddenly. The rat displays copulatory movements in the absence of the female and bends down to lick its penis in full erection; ejaculation ensues.

Results. These are summarized in the table. As is known[9-11], β^{1-24}-ACTH, injected into a lateral ventricle, induces a behavioural syndrome characterized by the great increase of the number of stretching and yawning movements and of spontaneous penile erections.

Naloxone caused a dose-related increase in the number of penile erections and a less pronounced increase in the number of stretchings and yawnings, which did, in fact, diminish at the highest dose.

In the rats treated with 0.5 µg of β^{1-24}-ACTH, naloxone had practically no effect. On the other hand, in the rats treated with 1 µg of ACTH, naloxone had little effect on penile erections but influenced the number of stretchings and yawnings, which increased greatly on dosage of 5 mg/kg and fell on dosage of 10 mg/kg.

Discussion. In our opinion, the results can be interpreted in 2 different ways. One is that naloxone, besides binding opiate receptors, also binds those for penile erection, stretching and yawning; the receptors for penile erection are always stimulated, while those for stretching and yawning are stimulated by low doses, inhibited by high doses: the same pattern has been observed for the anti-tremorine effect of MIF (MSH release inhibiting hormone, prolylleucylglycine amide)[18]. The other possibility is that naloxone does not in fact bind the receptors for penile erection at all, and that only at the highest dose does it bind those for stretching and yawning, thereby blocking them. The increase in the number of penile erections observed following the injection of naloxone might be explained by the fact that naloxone binds the opiate receptors and possibly other receptors as well. Thus the molecules of ACTH and related peptides which are physiologically present in the hypothalamus, and which also have affinity for the opiate and other receptors, are available in a larger number for the activation of the behavioural syndrome. The latter hypothesis, however, cannot be supported by using hypophysectomized rats, since we have seen that corticotrophin-releasing factor (CRF) too, in hypophysectomized animals, is able to induce the same behavioural syndrome[11]. Curiously, stretchings and yawnings are among the signs of the opiate abstinence syndrome in many mammals[19] and endorphins depress sexual activity in male rats (G.L. Gessa, personal communication).

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Effect of naloxone on the behaviour of rats and on the syndrome induced by intraliquorally injected ACTH

Treatment (naloxone mg/kg, s.c.; ACTH µg/rat intraliquorally)	Penile erections[a] (mean ± SE)		Stretchings[a] (mean ± SE)		Yawnings[a] (mean ± SE)	
Saline	0.6 ± 0.33	(30)	1.9 ± 1.14	(60)	3.0 ± 5.14	(60)
N 1	1.3 ± 0.69	(50)	2.7 ± 1.75	(50)	1.8 ± 0.69	(60)
N 5	1.7 ± 0.61	(60)	5.2 ± 1.39	(70)	13.0 ± 4.27[d]	(90)
N 10	4.4 ± 1.43[c]	(80)	0		2.0 ± 1.04	(80)
ACTH 0.5	1.8 ± 0.44	(60)	13.06 ± 2.92	(100)	13.53 ± 2.74	(100)
ACTH 1	3.30 ± 0.71[c]	(90)	22.9 ± 5.67[c]	(100)	21.30 ± 5.57[c]	(100)
N 1 + ACTH 0.5[b]	1.9 ± 1.26	(40)	11.30 ± 2.88[d]	(80)	12.20 ± 3.12[c]	(80)
N 5 + ACTH 0.5[b]	1.8 ± 0.24	(70)	17.50 ± 5.48[d]	(100)	21.40 ± 5.40[d]	(100)
N 5 + ACTH 1[b]	4.55 ± 1.16[d]	(100)	45.50 ± 12.70[e]	(100)	54.90 ± 14.80[e]	(100)
N 10 + ACTH 1[b]	4.80 ± 1.39[d]	(100)	18.80 ± 5.22[d]	(100)	17.80 ± 5.60[d]	(100)

Groups of 10 rats. N = naloxone; ACTH = β^{1-24}-ACTH. [a] Scored during the 2 h following treatment. In parentheses the % of rats with the syndrome; [b] Naloxone was injected 30 min before ACTH; [c] $0.02 < p < 0.05$; [d] $0.01 < p < 0.02$; [e] $p < 0.001$.

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Effects of anaesthesia and chronic catheterization on circulating levels of prostaglandins (PGE_2 and $PGF_{2\alpha}$ in dogs[1,2]

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Summary. Chronic catheterization of aorta and inferior vena cava in dogs did not significantly affect circulating levels of prostaglandins (PGE_2 and $PGF_{2\alpha}$). Pentobarbital (30 mg/kg i.v.) anaesthesia produced a significant decrease in $PGF_{2\alpha}$.

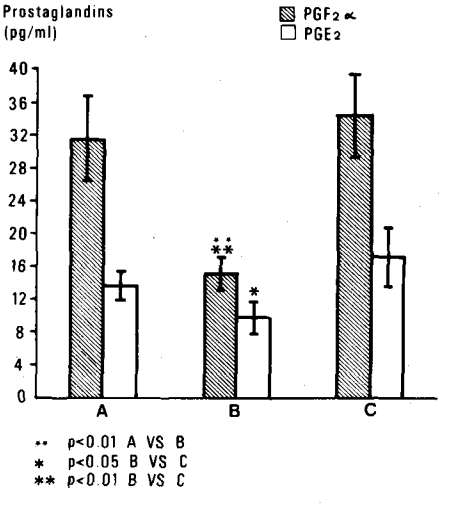
Normal circulating levels of prostaglandins have been difficult to determine due to limited sensitivity of many available assay procedures. Furthermore, values obtained in anaesthetized and conscious animals might differ, thus complicating comparison of the results reported by various authors. It was therefore felt necessary to evaluate the effect of anaesthesia on circulating levels of prostaglandins. The influence of chronic catheterization of blood vessels, a method often used when studying conscious dogs, was also determined.

Materials and methods. Nine mongrel dogs of average b.wt 23.8±1.3 kg (SEM) were used. Aortic and vena-caval catheters were inserted in aseptic conditions under pentobarbital anaesthesia (30 mg/kg) through iliac vessels, brought s.c. to the back of the animal and exteriorized at the level of intrascapular region. The catheters were filled with heparin (1000 U/ml) and flushed twice a week; in addition they were rinsed after each blood collection with 5 ml of normal saline followed by 1 ml (dead space of catheter) of diluted heparin solution.

A period of at least 1 week, and usually 2 weeks, preceded the first experiment (7-40 days). Intervals between experiments were no less than 3 days (3-26 days).

Collection of blood. About 10 ml of blood were drawn in a vacutainer containing EDTA (14 mg) either from direct puncture of a leg vein and/or from aortic and venous catheters. The first 3-5 ml of blood collected from the catheters were discarded. Hematocrit, determined in each dog, ranged from 37 to 45%. Immediately after collection, the blood was transferred in plastic tubes kept in ice and containing 0.1 ml of meclofenamic acid (500 µg/ml) in Tris hydrochloric acid 0.1 M, pH 8.4. The blood was centrifuged at 4500 rpm for 15 min at 4 °C. Supernatant plasma was collected without contamination by white blood cells and platelets. The plasma was stored at −20 °C till subsequent radioimmunoassay (2-10 days after blood collection). Radioimmunoassay of prostaglandins (PGE_2 and $PGF_{2\alpha}$) was conducted following the technique of Dray et al.[6]. The values are expressed as pg/ml of plasma±SEM in the text and figure. Blood was collected before anaesthesia (A), during anaesthesia (15-30 min after injection of pentobarbital 30 mg/kg) (B) and after anaesthesia (3 h after injection of pentobarbital 30 mg/kg) (C).

Results and discussion. The mean values of PGE_2 and $PGF_{2\alpha}$ in 5 conscious uncatheterized dogs were 9±2.4 and 20.4±9.1 pg/ml, respectively. The levels of both prostaglandins were slightly but not significantly higher (12.2±1.9, 36.9±9.4 pg/ml) in 5 conscious catheterized dogs. The levels of PGE_2 and $PGF_{2\alpha}$ in the plasma obtained from these 5 catheterized dogs were similar, whether it was collected by direct venous puncture (12.2±1.9, 36.9±9.4 pg/ml) or from the chronically implanted venous catheter (14.35±2.9, 28.2±6.7 pg/ml). Although there were wide variations in values of PGE_2 (27.4±12.95 pg/ml) and



Peripheral venous levels of prostaglandins before (A), during (B) and after anaesthesia (C) with pentobarbital 30 mg/kg i.v. (n = 13).