EFFECT OF MORPHINE AND NALOXONE, INJECTED INTO PREGNANT RATS ON THE ADRENALS AND TESTES OF THE OFFSPRING

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Opioids are involved in the regulation of the gonads and adrenals in man and animals [2, 4, 9, 12]. Opioid peptides and their receptors appear in the developing organism during the last third of embryogenesis [10], and at the times of ontogeny when critical stages of formation of the adrenal and gonadal systems take place they are already capable of influencing their endocrine function [2, 5, 12]. Action directed toward the endocrine system during a critical period can modify its activity for a long period of life [1, 3].

These facts suggest that morphine, a stimulator of opioid receptors, and naloxone, a blocker of these receptors, if injected into a pregnant rat, would have a prolonged effect on the testes and adrenals of the offspring. The study of this problem, directed toward elucidation of the opioid regulation of ontogeny of the endocrine glands, is also of definite practical interest because of the possible taking of opioids by women.

EXPERIMENTAL METHOD

Female Wistar rats were given subcutaneous injections of morphine (10 mg/kg; Biogal, Hungary), naloxone (10 mg/kg; Sigma, USA), in physiological saline, or physiological saline alone, in equivalent volumes (0.5 ml) daily from the 15th through the 18th days of pregnancy at 7 a.m, and 7 p.m. Some of the female rats were left intact. The relative weight of the testes and adrenals and hormone levels in blood taken after rapid removal of the animals from their cages, were determined in the offspring of these female rats at birth and at the age of 9 and 16 days and 2 months. Stress was ind by placing the 2-month-old animals for 1 h in plastic constraining cylinders. The blood testosterone level was determined by radioimmunoassay (DPC, France) and corticosterone by the competitive protein binding method [11].

EXPERIMENTAL RESULTS

The size of the litter and the mean weight of the young rats showed no significant change as a result of the procedures which the female rats underwent during pregnancy. The relative mass of the testes of newborn rats fell after injection of morphine or physiological saline into their mothers (Table 1). The blood testosterone level of young rats aged 9 and 16 days was low and did not differ at the two ages, so that the results could be pooled, The blood testosterone level in rats aged 9-16 days, whose mothers had received an injection of morphine or physiological saline, was significantly lower than the intact or naloxene groups.

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TABLE 1. Relative Weight of Testes and Adrenals and Blood Hormone Levels of Rats after Prenatal Exposure to Morphine or Naloxone (M ± m)

Group of animals	Age of animals			
	newborn	9 days	16 days	2 months
	Testes, mg/100 g body weight			
1	91±3 (17)	194±4 (11)	377+21 (6)	1172±34 (11)
2	68±5 (10)[1]	173±4 (14)[1]	342±8 (7)	1248±27 (10)
3	77±5(9)[1]	186±5 (7)	354±12 (5)	1308±28 (11)[1]
4	88±3(6)[2]	184±5 (13)	350±5 (4)	1258±36 (7)
	Testosterone, nM			
1	2,50±0,73 (14)			18,6±4,0 (9)
2	0,24±0,10 (14)[1]			20,8±4,3 (11)
3	0,76±0,28 (11)[1]			8,3±2,1 (9)[1,2]
4	2,57±0,76 (15)[2,3]			16,8±4,0 (10)
	Adrenals, mg/100 b body weight			
1	37,8±1,4 (31)	21,3±0,9 (17)	31,8±1,3 (11)	20,1±0,9 (11)
2	43,7±1,4 (25)[1]	22,2±0,6 (23)	32,1±0,9 (13)	22,1±0,8 (10)
3	44,0±1,2 (16)[1]	21,7±0,8 (12)	38,6±2,3 (8)[1,2]	22,4±0,5 (11)[1]
4	48,8±1,7 (9)[1,2,3]	22,6±0,9 (19)	29,9±1,4 (10)[3]	23,7±1,1 (8)[1]
1	100±7 (35)	12,1±2,9 (12)	66±10 (11)	71±27 (4)
2	132±14 (22)[1]	9,5±1,2 (22)	72±8 (12)	82±45 (5)
3	125±23 (16)	23,1±3,6 (4)[1,2]	82±13 (8)	114±27 (5)
4	119±22 (9)	12,3±2,2 (20)[3]	53±6 (9)[3]	112±45 (5)

Legend. Number of animals given in parentheses. Nos. of groups of the same age, differences with which are significant. Groups of animals: 1) intact rats, 2) with prenatal injection of physiological saline, 3) of morphine, 4) of naloxone. For each group, and for testosterone, values in first 3 age groups (newborn, 9 and 16 days) are equal.

Injection of physiological saline into a pregnant rat is a stressor for the animal, capable of activating the opioid systems of the fetuses [2]. During stress in pregnant animals the blood testosterone level of their fetuses falls [2, 3, 12], and this can be prevented by blocking the opioid receptors [12]. The protective action of naloxone, a blocker of opioid receptors, is manifested during postnatal development. In animals with prenatal injection of this drug, unlike those receiving physiological saline, no decrease was observed in the relative weight of the testes during the 1st day after birth or in the testosterone level at the age of 9-16 days compared with intact rats. Morphine had a similar action with prenatal stress, but it lasted longer. At the age of 2 months, the offspring of pregnant rats receiving physiological saline had the same testosterone levels as intact animals, but the hormone level of males of the morphine group was lower at this age than in the control rats.

Inhibition of the endocrine function of the testes is not a simple reflection of the effect of morphine and prenatal stress on the growth of the gland. At the age of 9 days, negative correlation ($r = -0.620$, $p < 0.05$) between the hormone level and weight of the testes was observed in these animals, but in rats of the morphine group aged 2 months, the testosterone level in the blood was lower than in intact males, but the relative weight of the glands was increased. These mutual relations between growth of the gland and formation of its endocrine function may be connected with the ability of opioids to inhibit steroid synthesis in the testis [7, 12], secretion of pituitary gonadotropic hormones [2, 4, 7], and the presence of feedback in the endocrine system.

The relative weight of the adrenals of the newborn offspring of all groups of rats receiving injections during pregnancy was increased compared with intact young rats (Table 1). The effect of morphine and prenatal stress on the adrenals was opposite to their inhibitory action on the testes. The opposite relationship between the glands also was manifested during normal development. Between the 1st and 9th days of life the testes increased in size but the relative mass of the adrenals decreased. The relative mass of the testes and adrenals of intact animals 9 days old correlated negatively ($r = -0.501$, p [illegible]01). Blockade of the opioid receptors disturbed this relationship. In newborn animals naloxone increased the mass of [illegible] glands compared with physiological saline, but in animals of the naloxone group aged 9 days positive correlation (r [illegible].657, $p < 0.05$) was observed between the relative mass of the testes and adrenals.

The blood corticosterone level of the newborn rats and the relative mass of the adrenals of the offspring aged 2 months of pregnant rats receiving injections during pregnancy had a tendency to rise compared with the intact animals. On the 9th day after birth, at a time of weakened functional activity of the adrenals [1], the corticosterone level was lower than

at the preceding and succeeding times of ontogeny. After this age, rats whose mothers had received naloxone or physiological saline by injection during pregnancy did not differ from intact rats in their corticosterone level.

Rats of the morphine group had a higher blood corticosteroid level at the age of 9 days and a higher relative mass of their adrenals at the age of 16 days than in the other animals. Animals with prenatal exposure to morphine also had a higher corticosterone level than rats whose mothers had received naloxone, on the 16th day of life, and a stronger hormonal response to the stressor at the age of 2 months. After limitation of movement of the animals the blood corticosterone level of the males of the morphine group increased to 698 ± 59, but in the naloxone group to only 455 ± 69 nM ($p < 0.05$). Intact rats and those receiving physiological saline had blood corticosterone levels during stress of 553 and 623 nM respectively. Acute administration of morphine activates the adrenal system of adult animals [6, 8, 9]. Injection of morphine into pregnant rats raises the blood ACTH and corticosteroid levels of the fetuses [5] and, judging from our own data, has a prolonged activating effect on the adrenals of the offspring.

Thus morphine, when injected into a pregnant rat, causes prolonged inhibition of the testes and stimulation of the adrenals of the offspring. Stress during a pregnancy also inhibits the testes of neonatal animals. Opioid receptor blockade by naloxone prevents the effects of prenatal stress on the testes, but, under these circumstances, disturbs the correlation observed between testes and adrenals during ontogeny.

LITERATURE CITED

1. E. V. Naumenko, N. N. Dygalo, and L. N. Maslova, Ontogenetic and Genetic-Evolutionary Aspects of the Neuroendocrine Regulation of Stress [in Russian], Novosibirsk (1990), pp. 40-55.
2. V. Rode, T. Okava, F. Shtal, et al., Ontogenetic and Genetic-Evolutionary Aspects of the Neuroendocrine Regulation of Stress [in Russian], Novosibirsk (1990), pp. 28-40.
3. G. T. Shishkina, Ontogenetic and Genetic-Evolutionary Aspects of the Neuroendocrine Regulation of Stress [in Russian], Novosibirsk (1990), pp. 96-106.
4. M. S. Blark, A. Fabbri, K. J. Catt, and M. L. Dufau, Endocrinology, **118**, 2097 (1986).
5. A. N. Brooks and J. R. G. Challis, J. Endocrinol., **119**, 389 (1988).
6. J. C. Buckingham and T. A. Cooper, Neuroendocrinology, **38**, 411 (1984).
7. T. J. Cicero, L. O'Connor, B. Nock, et al., J. Pharm. Exp. Ther., **248**, 256 (1989).
8. A. Grossman, J. Endocrinol., **119**, 377 (1988).
9. L. Koranyi and E. Endroczi, Acta Physiol. Hung., **62**, 75 (1983).
10. J. McDowell and I. Kitchen, Brain Res. Rev., **12**, 397 (1987).
11. B. E. P. Murphy, J. Clin. Kndocr., **27**, 939 (1967).
12. I. L. Ward and O. B. Ward, Handbook of Behavioral Neurobiology, ed. by N. Adler, D. Pfaff, and R. W. Goy, Vol. 7, New York (1985), pp. 77-98.