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0014-4754/85/030404-04$1.50 + 0.20/0

Selective effects of gonadal steroids on the response of peripheral serotonin receptors

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Summary. The maximal contraction provoked by serotonin (5-HT) in isolated stomach strips of adult rats, a functional index for peripheral 5-HT receptors, was sexually differentiated, androgen-sensitive, and estrogen refractory. This is at variance with the reported sensitivity of central 5-HT receptors to estrogen.
Key words. Rat, stomach strips, isolated; serotonin receptors, peripheral; androgen-sensitive contraction; estrogen-refractory contraction.

There is evidence that brain 5-HT receptors are modulated by ovarian hormones[1]. This finding is consistent with the existence of steroid binding sites in discrete regions of the brain[2]. There is also an apparent[3], though unlikely[4] similarity between receptor sites which bind ^{3}H-5-HT in the brain, and those[5] which putatively trigger the 5-HT induced contraction in the rat stomach fundus preparation. In fact, several compounds with high binding affinity at 5-HT central receptors would also markedly antagonize the response to 5-HT or act as serotonergic agonists on the isolated fundus strip[3,4]. Furthermore, the rank order of displacing potencies of 5-HT antagonists on ^{3}H-5-HT binding is similar in rat brain and gut membranes[6]. It was, therefore, of interest to investigate whether chronic exposure of rats to gonadal steroids would influence the isolated stomach strip response to 5-HT, as was observed with brain serotonergic binding sites[1].

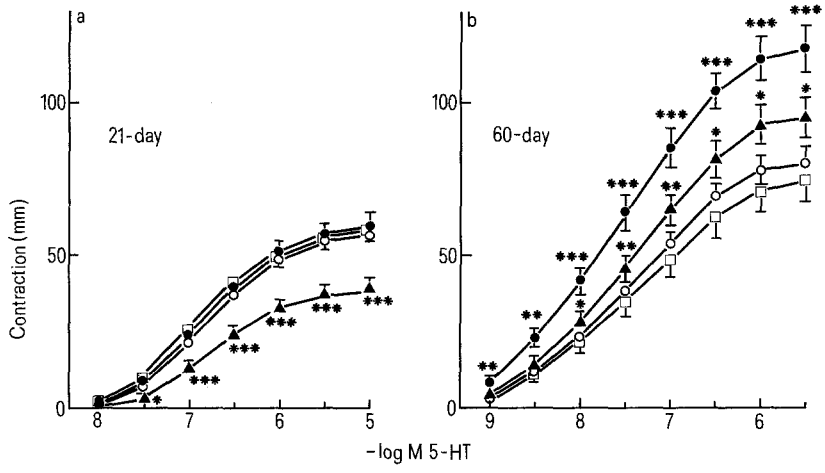
Materials and methods. Newborn male (M) and female (F) albino Charles River CD rats were used. The experimental schedule of the main experiment[7] was intended to masculinize the brains of neonatal F with androgen administration, or to interrupt the masculinization process of noncastrated M with estrogen. For this purpose, M and F pups were given, respectively, a single s.c. dose of 17β-estradiol valerate (Sigma Chemical Co.; E_2; 500 µg in 0.05 ml of corn oil), or testosterone enanthate (Sigma; TS; 270 µg) within 24 h from birth. Rats then received approximately weekly injections of decreasing doses of steroids: 200 µg E_2 or 100 µg TS at days 5, 10 and 17; 100 µg E_2 or 10 µg TS on day 24; 1 µg E_2 or TS at days 34 and 44, and 0.5 µg E_2 or TS at 50 days of age. Control pups received corn oil on the same days as the steroid-treated rats. All animals were sacrificed at 21 or 60–61 days of age. Adult, control females were in late metestrus or diestrus, as assessed with vaginal smears. The stomach was dissected soon after sacrifice, and two strips were cut from the fundus according to a slight modification[8] of the classical Vane's method. The mucosa was carefully ablated, and one strip was set up in a 10-ml organ bath at 37 °C containing Tyrode solution bubbled with O_2. Composition of the Tyrode solution was (g/l): NaCl 8; KCl 0.2; $CaCl_2$ 0.2; $MgCl_2$ 0.1; $NaHCO_3$ 1; NaH_2PO_4 0.05, and glucose 1. One end of the strip was connected to a constantly loaded isotonic lever, and 5-HT-induced contractions were registered on a slowly rotating, smoked kymograph. The cumulative dose-response technique was used, and pD_2 values were calculated[9]. A pD_2 value indicates the 'affinity' of 5-HT for its receptors, and is defined as the negative logarithm of the molar concentration of 5-HT producing a contraction 50% of the maximal one in that system[10]. Duplicate to quadruplicate dose-response curves were made for each strip, and the average pD_2 was calculated for each individual experiment. Statistical significance of differences was assessed with Student's t-test.

Results and discussion. The maximum effect of 5-HT increased with age; over the same time, the dose-response curves at 21 days were shifted far to the right on the dose axis (fig., a) thus indicating neonatal hyposensitivity to 5-HT[11]. Neonatal pD_2 values were consequently 5-fold lower than those in control and hormone-manipulated adults (table). The pD_2 values at both ages were neither sexually differentiated, nor were they affected after chronic administration of E_2 to males or TS to females (table). There was, however, a consistent sex-related difference in the maximal effect of 5-HT, inasmuch as the peak

The influence of neonatal and prepubertal chronic administration of estradiol (E_2) and testosterone (TS), age and sex on the receptor affinity* for 5-HT in the isolated rat stomach fundus preparation

Sex	In vivo treatment	Days of age 21 pD_2	n_1/n_2	60 pD_2	n_1/n_2
Male	Corn oil	6.85 ± 0.07***	15/43	7.46 ± 0.08	32/102
Female	Corn oil	6.88 ± 0.08***	16/40	7.60 ± 0.06	35/110
Male	E_2	6.92 ± 0.09**	13/28	7.40 ± 0.10	27/77
Female	TS	6.77 ± 0.09***	12/28	7.55 ± 0.08	30/93

*The pD_2 values given are means ± SE of n_1 experiments on fundus strips; pD_2 values for individual experiments were calculated from 2–4 dose-response curves. n_2 = total number of cumulative curves. **$p < 0.005$; ***$p < 0.001$, compared to 60-day-old counterparts.



Cumulative log dose-contraction curves for 5-HT on the isolated rat stomach fundic strip preparation at 21 (a) and 60 (b) days of age. Newborn male rats received corn oil (○–○) or decreasing doses of estradiol (E_2) (□–□); newborn females received corn oil (●–●) or decreasing doses of testosterone (TS) (▲–▲) from birth up to 17 or 50 days of age. The number of experiments/strip ranged from 12 to 35, each consisting of 2–4 cumulative dose-response curves. *$p < 0.05$; **$p < 0.025$ or < 0.01; ***$p < 0.005$ or < 0.001, (○–○) vs (●–●), or (●–●) vs (▲–▲).

contraction was by 46% ($p < 0.001$) greater in strips obtained from adult females, compared to male rats (fig., b). The administration of E_2 to males did not alter the 5-HT contraction, whereas TS to females, a process known to induce masculinization, did consistently decrease the peak contraction in adult and 21-day-old rats (by 192, $p < 0.05$, and by 36%, $p < 0.001$), respectively (fig., a and b).

The present study has shown that, unlike central 5-HT binding sites, peripheral serotonergic receptors were not influenced by a chronic administration of E_2, whereas they were affected by TS. Furthermore, the response of stomach strips to 5-HT was sexually differentiated and androgen-dependent. In fact, the maximum effect of 5-HT, which is normally greater in the smooth muscle of adult female rats than in that of males, was inhibited by a neonatal and prepubertal exposure of female pups to decreasing doses of TS. A role of androgen in modulating the 5-HT contraction was also supported by the absence of sex-related differences at 21 days, an age when the levels of serum TS are similarly low in both sexes[12]. Conversely, the peak effect of 5-HT was smaller at the time when circulating levels of TS are markedly higher in adult males, compared to female rats[12].

It is well-known that both androgen and estrogen steroids in vitro exert a nonspecific spasmolytic effect on contractions provoked by 5-HT and other agonists, as well as stimulatory procedures in smooth muscle preparations taken from accessory genital tissues and the gastrointestinal tract[13–17]. Present results, however, show that there was a steroid-specific influence on the response to 5-HT after in vivo exposure of rats to gonadal hormones. The functional relevance, if any, of this selectivity and of the greater response of the female stomach to 5-HT is difficult to ascertain. Maybe this is just one facet of the well-known sexual differentiation in central and peripheral monoaminergic neurotransmission[18]. The pD_2 values were similar in both sexes at both ages, and were not affected by hormone manipulations. Since a pD_2 value results from the measurement of the relative dose of agonist needed to induce a certain fraction of its potential maximum effect, it represents an indirect measure of the receptor affinity for interacting molecules[10]. Alterations in the maximal contraction may either reflect changes in the number of agonist-receptor complexes formed, or/and in the activated receptor effector coupling, thus representing an approximate functional index for changes in the receptor density.

In conclusion, the present results show that putative serotonergic receptors in the rat stomach can functionally adapt to alterations in androgen, but not estrogen homeostasis, without corresponding alterations in the receptor affinity for 5-HT. This is at variance with central, estrogen-sensitive 5-HT receptors[1]. It is unfortunate that as yet there are no clear-cut functional correlates for ^{3}H-5-HT binding in the brain[19] which would enable us to ascertain whether 5-HT recognition sites and the central response they eventually trigger may respond differently to hormone challenges.

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