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Hyposensitivity to 5-hydroxytryptamine in the isolated stomach fundus of the newborn rat—II. Amphetamine and receptor deficit

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We have previously shown the occurrence of a reduced affinity (by a factor of −23) for 5-hydroxytryptamine (5-HT) in the isolated gastric fundus of the newborn rat.[1] Intrinsic minor contractility of the smooth muscle not yet fully developed cannot be entirely accepted as only reason of the hyposensitivity. Other more specific reasons are possible: among them a 5-HT receptor *deficit*, in the newborn animal. This hypothesis is examined in the present paper.

The investigation is based upon the assumption that amphetamine acts directly on the 5-HT receptor[2–4] in several isolated organ preparations, among them rat fundal strips.[3] Assuming that the concept of identical receptors for the two amines is correct, one would predict neonatal hyposensitivity also to amphetamine. This reduced affinity should be similar to that evidenced with 5-HT, and might support the 5-HT receptor deficit hypothesis. Both DL- and D-amphetamine are tested in the present experiments, to control possible differences between the racemic drug and the optical isomer with higher direct action on the 5-HT receptor.[3]

MATERIALS AND METHODS

Ninety-five Wistar albino rats of either sex were used. The gastric fundus preparations were described in detail in a previous paper.[1] Cumulative log dose–response curves were obtained with the drugs. Significance was tested on the pD_2 (affinity[6]) mean values, by Fisher's-F-test.[7]

The following drugs were employed: 5-hydroxytryptamine creatinine sulphate (Merck, Germany) D-amphetamine sulphate (Merck) and DL-amphetamine sulphate (Merck). Doses are expressed as base.

RESULTS AND DISCUSSION

Table 1 gives the affinity values for DL-amphetamine, and Table 2 the affinity for D-amphetamine,

TABLE 1. NEONATAL AFFINITY (pD_2) AND RELATIVE SENSITIVITY TOWARDS DL-AMPHETAMINE AT VARIOUS AGE DAYS

Age	pD_2*†	Est/org‡	Relative sensitivity§	f	P‖
Adult	4·78 (±0·108)	134/50	1·00		
1 day	4·61 (±0·243)	27/10	~1·00	2·978	n.s.
9 days	4·88 (±0·112)	73/19	~1·00	2·580	n.s.

* pD_2 (affinity): negative logarithm of the concentration of agonist required to produce 50 per cent of the maximal response obtained in the system.

† (±): 95 per cent confidence limits for the mean.

‡ Est/org.: number of cumulative dose–response curves/number of organs used.

§ Relative sensitivity expressed as factor of the affinity in the adult animal (=1·0; inverse log. scale).

‖ Significance levels of the neonatal affinity in comparison to that of the adult.

TABLE 2. NEONATAL AFFINITY (pD_2) AND RELATIVE SENSITIVITY TOWARDS D-AMPHETAMINE AT VARIOUS AGE DAYS

Age	pD_2*†	Est/org‡	Relative sensitivity§	f	P‖
Adult	4·71 (±0·117)	55/15	1·00		
1 day	4·00 (±0·051)	83/13	−5·20	395·67	<0·001
9 days	4·60 (±0·202)	64/10	~1·00	<1	n.s.

* pD_2 (affinity): negative logarithm of the concentration of agonist required to produce 50 per cent of the maximal response obtained in the system.

† (±): 95 per cent confidence limits for the mean.

‡ Est/org.: number of cumulative dose–response curves/number of organs used.

§ Relative sensitivity expressed as factor of the affinity in the adult animal (=1·0; inverse log. scale).

‖ Significance levels of the neonatal affinity in comparison to that of the adult.

in 1 and 9 day-old rats. The 9th day was chosen because at about this age (half of the rat neonatal-period), the enzyme activities are approaching the adult levels. With DL-amphetamine, no statistically significant variation of the neonatal affinity occurs, in comparison to that of the adult animal. The results with D-amphetamine show in the 1 day-old animal, a weak decrease in affinity, by a factor of 5·2 *in minus*. This factor is practically identical to that obtained with the cholinergic drug furtrethonium (HFur)[1] on same preparation of newborn rat. A possible explanation is that immature smooth muscle and experimental factors interfere with the affinity of D-amphetamine, as well as with HFur. Undoubtedly the factor of −5·2 is clearly smaller than that related to 5-HT (−23).

The results show evidence that amphetamine, unlikely 5-HT, binds readily to its receptor system. The different behaviour of the neonatal affinity towards 5-HT and amphetamine support the concept

that immature smooth muscle plays a minor role in the 5-HT neonatal hyposensitivity. There is indirect evidence that the hypothesis of receptor deficit for 5-HT cannot be supported. If receptor deficit were indeed present in the origin of 5-HT hyposensitivity, then a similar decrease in affinity towards amphetamine would be expected. The problem whether 5-HT and amphetamine act on the same receptor is now being investigated.

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Alanosine and hadacidin—Comparison of effects on adenylosuccinate synthetase*

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ALANOSINE [L(–)2-amino-3-nitrosohydroxylamino propionic acid], originally obtained from *Streptomyces alanosinicus* (ATCC No. 15710), has inhibitory activity against a number of viruses *in vitro* and *in vivo*, and induces regression of a transplanted fibrosarcoma.[1] Studies of its mode of action in an ascites tumor system[2] *in vivo* showed that it inhibits the synthesis of RNA adenine from formate-^{14}C and from glycine-^{14}C with no appreciable reduction of the rate of RNA guanine synthesis. The incorporation of preformed adenine-^{14}C into RNA purines is not inhibited. A similar pattern of activity was observed in a microbial system.[3] In addition, the incorporation of aspartate-^{14}C into microbial RNA pyrimidines is inhibited while the incorporation of uridine-^{3}H is enhanced. The fact that inhibition by alanosine of the growth of *Candida albicans* was antagonized by aspartate led to the tentative suggestion that the drug depresses the activity of adenylosuccinate synthetase and aspartate transcarbamylase.

Hadacidin (*N*-formyl hydroxyaminoacetic acid), an antibiotic from *Penicillium frequentans*, has an action mode similar to that of alanosine in that it inhibits the incorporation of formate and glycine into adenylic acid with no inhibition of guanylic acid formation.[4] Subsequent studies of a partially purified adenylosuccinate synthetase revealed the enzyme to be quite sensitive to hadacidin; at an

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