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BRAIN SEROTONIN DEPLETORS AND ADRENOCORTICAL ACTIVATION*

P. PREZIOSI, U. SCAPAGNINI and G. NISTICO'

2nd Chair of Pharmacology, Faculty of Medicine, University of Naples, Italy

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Abstract—*p*-Chlorophenylalanine, prenylamine and α-methyldopa are brain serotonin depletors. The first acts in an elective manner; if administered to the rat by the routes and at doses which cause a marked reduction in the content of cerebral 5-hydroxytryptamine, it does not bring about adrenocortical activation either at the time when there is maximum depletion of brain serotonin or later. The adrenocortical activation provoked by prenylamine given i.v. may be related to the acute hypotension which the drug produces. Data obtained did not corroborate the importance of brain serotonin depletors in the liberation of CRF and consequent activation of the pituitary-adrenal axis.

ATTENTION has been focused on the hypothalamic content of 5-hydroxytryptamine (5-HT) and the activation of the pituitary-adrenal axis.[1] In particular, when the cerebral content of 5-HT is reduced by reserpine to less than 50 per cent the basal values, the mechanism which leads to the liberation of CRF-ACTH would be set off. Iproniazid, a monoamine oxidase inhibitor able to antagonise the reserpine induced reduction of cerebral 5-HT, has been shown to markedly inhibit the adrenal stimulation provoked by the alkaloid.[2–4] This same iproniazid, in both man[5] and animals,[6] has been found to be able to antagonise the adrenal activation provoked by other stress agents. There are, however, contradictory opinions concerning this property of iproniazid[4,7,8] and of another MAO-inhibitor, pargyline.[9]

An inhibitory action on stress induced ACTH secretion may also be held by another MAO-inhibitor, α-ethyltryptamine,[7,9] but with a mechanism which is not related to modifications affecting the hypothalamic content of biogenic amines.[9] We have been able to demonstrate that the "activation" of cerebral 5-HT by reserpine, is not responsible for the stimulation of CRF and ACTH release induced by the alkaloid.[10]

It has also been shown that the glucocorticoids are indispensable in the maintenance of a normal quantity of the bound form of 5-HT in the brain.[11] Toh[12] has shown that the cerebral content of 5-HT becomes reduced under conditions which are also known to determine an adrenal activation (stress due to cold). Other investigations have, however, supplied conflicting results.[13] As a consequence of stress, the reduction in the hypothalamic content of 5-HT, both in the free and bound forms, would probably be due to a reduction in high energy phosphate compounds. For example, cerebral ATP would be noticeably reduced in the hypothalamus under influences of stress.[14,15]

We have wanted to see if brain 5-HT depletors, whether specific (*p*-chlorophenylalanine, *p*-CPA:[16,17] or less specific although still able to deplete (prenylamine,[18–20

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α-methyldopa[21,22]) norepinephrine or other biogenic amines* were able to activate the adrenal cortex in such doses as to determine an intense depletion of serotonin. The eventual adrenocortical activation was noticed by determining the blood levels of corticosterone both at the time of maximum serotonin depletion (*p*-CPA 24–72 hr,[17] prenylamine 1–10 hr,[18–20] α-methyldopa 1–5 hr[21,22,25–27]) and at times distant from these.

METHODS AND MATERIALS

Chemicals

Chemicals used were: *p*-chlorophenylalanine (*p*-CPA) solubilized as described by Kenneth Koe and Weissman,[17] *N*-3'phenylpropyl-(2')-1,1-diphenyl-propyl-(3)-amine (prenylamine) as 5% gluconate in H_2O, and α-methyl-3,4-dihydroxyphenylalanine (α-methyldopa), aqueous solution in 200 mg vials. The reagents for the dosage of plasma corticosterone are those used in the method of Silber *et al.*[28] Methylene chloride was always purified by means of distillation as described by Mattingly.[29]

Animals

Male rats of the Wistar–Morini strain with body weight between 200–230 g were used. Fifteen days prior to the beginning of the experiment, the animals were placed in groups of three in makrolon cages for rats (cm 42 × 27 × h 15). Their environment was thermoregulated at 22°.

Routes of administration and dosage

p-CPA was given i.p. at a dose equivalent to 316 mg/kg. This dose was found able to induce an intense depletion of cerebral serotonin, with a peak action between the 24th and 72nd hr after its administration.[17] Prenylamine was injected as follows: (a) i.v. (dorsal vein of the penis) in doses of 5 mg/kg which determined a marked depletion of serotonin, norepinephrine and dopamine between the 1st and 6th hr of its administration, with a subsequent return to normal after the 24th hr,[20] and, (b) s.c. in a dose of 100 mg/kg which provoked a rapid fall (2–8 hr) and a slow return (72–96 hr and more) in brain serotonin and norepinephrine levels.[19] Alpha-methyldopa injected i.p. in the mouse,[21,22,26,27] rat,[21] guinea pig[21] and dog[25] in doses of 200 mg/kg provoked brain serotonin depletion (as well as norepinephrine and dopamine depletion). This dose given i.p. was used in our studies.

Withdrawal of blood samples

Blood taken from the decapitated animals was collected in heparinized containers, centrifuged and the plasma immediately frozen if the determination was not done at once.[1] Blood samples were taken 2, 6, 12, 24, 72, 96 and 168 hr after the administration of *p*-CPA, 2, 6, 24 hr after an i.v. injection and 6, 24, 48, 168 hr after a s.c. administration of prenylamine, and 2, 6, 24 hr after the α-methyldopa was given (i.p.). These time periods correspond to the peak in brain serotonin depletion attained with the above mentioned drugs and their eventual return to normal values.

Plasma corticosterone

The plasma levels of corticosterone were determined according to Silber *et al.*[28] with minor modifications. The readings were taken from the Aminco–Bowman

* The depletion of these amines may occur almost exclusively with prenylamine.[23,24]

spectrophotofluorometer. The excitation was performed at 470 mμ with maximum fluorescence occurring at 530 mμ.

The residual fluorescence from plasma of adrenalectomized animals with same weight and sex (μg/100 g ml plasma: 5·5 ± 0·02) was subtracted from each value arrived at. For this research, 20 rats were used which had been adrenalectomized three days before the plasma corticosterone determination. In preliminary experiments we have also ascertained the fact that the intramuscular administration of a strong dose of ACTH (10 U/kg) led to plasma corticosterone levels equal to (μg/100 ml) 59·7 ± 5·3 (data on 10 animals).

RESULTS AND DISCUSSION

p-Chlorophenylalanine does not determine significant variations of plasma corticosterone levels with respect to the controls. The increase which is noted between the 24th and 48th hr (a time in which the peak effect of brain serotonin depletion occurs) is not statistically significant. The plasma corticosterone values of rats treated with α-methyldopa are inferior to those of the controls, especially 2 hr after its administration. However, 2 hr after the i.v. administration of a dose of prenylamine there is an increase in plasma corticosterone equal to 37 per cent of the controls, a finding statistically significant. This is followed by a marked reduction (at the 6th hr,)which is also statistically significant. There is a subsequent return to normal at the 24th hr. Animals i.v. injected with physiologic solution show no increase in plasma corticosterone levels after the second hour. This does not result statistically different from the controls. Rats which received a subcutaneous dose of prenylamine did not show noteworthy modifications in plasma corticosterone content (Table 1). In the majority of cases, however, values were found to be less than those of the controls.

TABLE 1. ACTION OF BRAIN SEROTONIN DEPLETORS ON PLASMA CORTICOSTERONE LEVELS IN THE RAT

Treatment	Dose mg/kg	Route of drug administration	Hours after administration	Plasma levels of corticosterone mean values* μg/100 ml ± S.E.M.	P
(Control)	—	—		20·8 ± 2·4	
Saline	2 ml/kg	i.v.	2	16·1 ± 3·6	N.S.
p-Chlorophenylalanine	316	i.p.	2	21·1 ± 4·2	N.S.
p-Chlorophenylalanine	316	i.p.	6	18·6 ± 2·4	N.S.
p-Chlorophenylalanine	316	i.p.	12	23·7 ± 3·1	N.S.
p-Chlorophenylalanine	316	i.p.	24	25·4 ± 2·9	N.S.
p-Chlorophenylalanine	316	i.p.	48	23·0 ± 2·2	N.S.
p-Chlorophenylalanine	316	i.p.	72	20·6 ± 3·1	N.S.
p-Chlorophenylalanine	316	i.p.	96	23·6 ± 2·7	N.S.
p-Chlorophenylalanine	316	i.p.	168	16·9 ± 3·8	N.S.
Prenylamine	5	i.v.	2	28·6 ± 2·8	< 0·05
Prenylamine	5	i.v.	6	12·0 ± 1·5	< 0·01
Prenylamine	5	i.v.	24	19·2 ± 3·7	N.S.
Prenylamine	100	s.c.	6	27·1 ± 3·3	N.S.
Prenylamine	100	s.c.	24	15·7 ± 2·9	N.S.
Prenylamine	100	s.c.	48	12·0 ± 3·1	< 0·01
Prenylamine	100	s.c.	168	15·4 ± 3·3	N.S.
α-Methyldopa	200	i.p.	2	14·9 ± 2·5	N.S.
α-Methyldopa	200	i.p.	6	17·9 ± 3·6	N.S.
α-Methyldopa	200	i.p.	24	19·1 ± 3·8	N.S.

* Obtained on 10 animals/group. Forty rats were used as controls.
P: probability (N.S.: *t* test > 0·05)

Of the brain serotonin depletors examined by us, neither those highly specific (*p*-CPA) which probably act by a mechanism of synthesis inhibition,[17] nor those partly aspecific though also active on norepinephrine or other biogenic amines present in nervous tissue, were able to determine an adrenocortical activation in doses which at the same time were able to provoke an intense depletion in brain serotonin content at those times in which such a depletion was most marked or successively there after. The increase obtained 2 hr after an i.v. administration of prenylamine is to be considered aspecific and related to the marked hypotension which the compound determines in the rat at the dose and route of administration indicated.[30] Therefore, our experimental data does not corroborate the hypothesis which stresses the importance of the hypothalamic depletion of serotonin in activating the adrenal cortex via CRF-ACTH. In such a case the corticotropic pituitary function would be different from those which interfere with ovulation.

The process of ovulation would then be inhibited through pharmacologically induced variations in the cerebral content of serotonin instead of catecholamines.[31] Recently Smelik[32] has also found that the hypothalamic depletion of monoamines is not essential for the ACTH-releasing effects of reserpine.

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