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Brain monoamines and adrenocortical activation

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1. The effect of a pharmacologically induced increase or depletion of brain monoamines (5-hydroxytryptamine, noradrenaline, dopamine) was investigated with respect to adrenocortical function.

2. No strict correlation was found between the depletion of brain amine stores induced by prenylamine or *p*-chlorophenylalanine and adrenocortical activation, even at times in which the peak effect of brain amine depletion occurs.

3. Restraint stress causes a manifest increase in brain 5-hydroxytryptamine while decreasing the cerebral noradrenaline and dopamine content. This stress strongly stimulates corticosterone secretion by the adrenals.

4. A monoamine oxidase inhibitor, nialamide, at a dose which causes an evident increase in brain amine concentrations, does not modify plasma corticosterone. At the same dose it was, furthermore, unable to prevent the adrenocortical stimulation induced by restraint stress.

5. Brain amine content does not seem to play an important part in the control of corticotrophin releasing factor in corticotrophin secretion by the pituitary gland. The relationship between hypothalamic monoamines and other neurohumours is discussed.

Several investigators have suggested that there is an important connection between the concentration of monoamines in the hypothalamus and the hypothalamic neurotransmitters which control the release of pituitary hormones. This conclusion was drawn from two types of observation. First, some drugs which decrease the concentration of brain amines were also found to cause variations in the function of the endocrine organs. Thus the release of the corticotrophin releasing factor (CRF) by a dose of reserpine which caused a 50% fall in the concentration of 5-hydroxytryptamine (5-HT) in the brain was described by Westermann, Maickel & Brodie (1962). The administration of iproniazid, an inhibitor of monoamine oxidase (MAO) to mice not only prevented the fall in brain amines but also the stimulation of the adrenal cortex caused by reserpine (De Schaepdryver & Preziosi, 1959). This effect has also been observed in the rat (Eechaute, Lacroix, Leusen & Bouckaert, 1962; Manca, Miele, Passarelli & Preziosi, 1963; Zoryan, 1965). Also, a chemically induced depletion of the brain noradrenaline (NA) can be accompanied by a pseudopregnancy caused by an increased release of luteotrophic hormone (Coppola, Leonardi, Lippmann, Perrine & Ringler, 1965). In addition, Coppola,

Leonardi & Lippmann (1966) have shown that the secretion of luteinizing hormone, which causes ovulation after the administration of pregnant mare serum gonadotrophin, can be blocked when the NA stores in the brain are reduced. These effects, if induced by reserpine, can be counteracted by monoamine oxidase inhibitors such as iproniazid or pheniprazine. Furthermore, the release of growth hormone (GH) which follows the secretion of growth hormone releasing factor induced by insulin hypoglycaemia can be completely blocked by reserpine and by other NA depletors (Müller, Sawano, Arimura & Schally, 1967).

The second type of observation is that when rats and mice are exposed to situations which cause stress there is a reduction in the concentration of biogenic amines in the brain (Sulser & Brodie, 1960; Toh, 1960; Barchas & Freedman, 1963; Maynert & Levi, 1964; Moore & Larivière, 1964; Gordon, Spector, Sjoerdsma & Udenfriend, 1966; Guarino, Rosencrans, Mendillo & De Feo, 1967; Corrodi, Fuxe & Hökfelt, 1968a; Corrodi, Fuxe & Hökfelt, 1968b; Welch & Welch, 1968a; Welch & Welch, 1968b). Such stressing conditions are always accompanied by activation of the adrenal cortex (Friedman & Ader, 1967; Yates, 1967). The possible correlation between the changes in the concentrations of monoamines in the brain and the activation of the adrenal cortex is not clear, however, and the present paper describes some experiments which were carried out to investigate whether a correlation exists between the concentration of NA, 3,4-dihydroxyphenylethylamine (dopamine, DA) and 5-HT in the brain and the concentration of corticosterone in the blood plasma. This index of adrenal cortical activation was measured both at the time of maximum drug induced depletion of brain amines (prenylamine: Juorio & Vogt, 1965, 1–12 hr; Obianwu, 1965, 1–10 hr; Schöne & Lindner, 1960, 1–10 hr; *p*-chlorophenylalanine (*p*-CPA): Koe & Weissman, 1966, 24–72 hr) and at times other than these.

Methods

Animals

Male adult rats of the Wistar-Morini strain with a body weight between 120–150 g were used. Fifteen days before the beginning of the experiment, the animals were placed in groups of three in Makrolon cages for rats (42 × 27 × 15 cm). The environmental temperature was maintained at 22° C. The animals were fed Purina laboratory chow supplemented with fresh vegetables and given water *ad lib.* All rats were fasted overnight before the treatment, when not otherwise specified.

Restraint stress

Rats which had been fasted for more than 16 hr were placed in a restraining jacket of fine mesh wire. Their paws, protruding through holes in the wire, were immobilized with adhesive tape. The jackets containing the rats were then suspended horizontally by means of rings from a suitable support.

Drugs

The following drugs were used: N-[3′phenylpropyl-(2′)]-1,1-diphenyl-propyl-(3)-amine (prenylamine gluconate, 5% in H_2O), *p*-chlorophenylalanine (*p*-CPA) solubilized as described by Koe & Weissman (1966), N-(2-benzylcarbamoylethyl)N^1-2-isonicotinyl hydrazine (nialamide). The latter drug was dissolved in distilled water

(0.5% aqueous solution). When drugs were used as salts, the doses were expressed in terms of drug base.

Routes of administration and dosage

Prenylamine was injected as follows: (a) intravenously (dorsal vein of the penis) in a dose of 5 mg/kg which causes a depletion of 5-HT, NA and DA in the brain between the 1st and 6th hour after administration, with a subsequent return to normal after 24 hr (Werdinius, 1967); (b) subcutaneously in a dose of 100 mg/kg which caused a rapid fall (2–8 hr) and a slow return (72–96 hr or longer) of brain 5-HT and NA concentrations (Schöne & Lindner, 1960).

p-CPA was given intraperitoneally in a dose of 316 mg/kg. This dose was found to induce a large depletion of cerebral 5-HT only, with a maximum effect between the 24th and 72nd hour after its administration (Koe & Weissman, 1966). Nialamide was injected by the intramuscular route in a dose of 50 mg/kg. Previous observations have shown that this dose increases the concentrations of 5-HT and NA in the brain (Leroy & Van der Schoot, 1962).

Collection of blood samples and dissection of brains

Blood was collected in vessels containing heparin after decapitating the animals. When the corticosterone estimations were not done immediately the plasma was frozen and stored at $-10°$ C (Westermann, Maickel & Brodie, 1962). Blood samples were taken 6, 24 and 168 hr after the administration of prenylamine, 6, 24, 48 and 72 hr after *p*-CPA was given. Rats treated with nialamide were killed 26 hr after the injection. Some of them were restrained for the last 6 hr of this period. Those animals which were stressed by immobilization only were killed after 6 hr of restraint. Two brains were pooled for the estimation of the monoamines.

Plasma corticosterone

The concentration of corticosterone in the plasma was estimated by the fluorescence method of Silber, Bush & Oslapas (1958). Methylene chloride was purified as described by Mattingly (1962). Fluorescence was measured using an Aminco-Bowman spectrophotofluorometer (excitation 470 mμ; fluorescence 530 mμ).

The residual fluorescence which was obtained with plasma of adrenalectomized animals of same weight and sex (5.5 ± 0.02 μg/100 ml. plasma) was subtracted from each value. For these experiments, twenty rats were used which had been adrenalectomized 3 days before. In preliminary experiments we have also ascertained that the intramuscular administration of a large dose of ACTH (10 u./kg) led to a plasma corticosterone concentration of 59.7 ± 5.3 μg/100 ml. (data from ten animals).

Monoamine estimation

The brain tissue was homogenized with 0.4 N perchloric acid (20 ml./g of tissue). NA and DA were extracted according to Laverty, Sharman & Vogt (1965) and adsorbed on a Dowex 50 W $\times$ 4 column. NA was eluted with 0.4 N HCl and DA with 2 N HCl. NA was determined fluorometrically after oxidation with $K_3Fe(CN)_6$ (von Euler & Lishajko, 1961), and DA according to Carlsson & Waldeck (1958). For extraction and estimation of 5-HT the fluorimetric method of Snyder, Axelrod & Zweig (1965) was used.

Blood pressure

In some experiments, arterial blood pressure was recorded after drug administration, either from the carotid artery by means of a mercury manometer, or from a tail artery using a Beckman continuous systolic monitor with a miniature pick up.

Results

Effects of prenylamine and p-*chlorophenylalanine on brain amines and on plasma corticosterone*

The results which were obtained with rats treated with prenylamine and *p*-CPA are summarized in Table 1. Both drugs caused a fall in the concentration of all of the monoamines. The maximum effects of prenylamine were seen at 2 hr (NA and DA) and 6 hr (5-HT) after the intravenous (5 mg/kg) injection and at 6 hr (NA, DA and 5-HT) after the subcutaneous (100 mg/kg) administration. 24 hr after the intravenous injection the concentrations of the monoamines were returning to normal and recovery was nearly complete 168 hr after the drug had been given subcutaneously. The plasma corticosterone was increased by about 40%, 2 hr after the intravenous injection and 6 hr after the subcutaneous injection of prenylamine. 6 hr after intravenous and 24 hr after subcutaneous administration of prenylamine, however, when the cerebral concentration of monoamine was still reduced, the plasma corticosterone was, in fact, somewhat lower than normal. In control experiments the intravenous injection of 0.9% sodium chloride solution did not cause an increase in the plasma corticosterone after 2 hr. *p*-CPA (316 mg/kg caused a significant fall in the concentration of NA and DA as well as of 5-HT. This effect lasts for up to 72 hr, the maximum effect occurring between 24 and 48 hr after the injection. In spite of a fall of 45% in the concentration of 5-HT and falls of 23% and 12% in the concentration of NA and DA 24 hr after the administration of *p*-CPA, the concentration of corticosterone in the plasma was not increased.

Restraint stress and inhibition of monoamine oxidase

Results which were obtained on rats, immobilized for 6 hr before decapitation, are summarized in Table 2. Restraint alone doubled the plasma corticosterone concentration. Simultaneously there was a rise of 10% in the brain concentration of 5-HT and a fall of 17% and 15% of the brain concentrations of noradrenaline and dopamine respectively. The brains of rats which received an intramuscular injection of nialamide (50 mg/kg) 26 hr before decapitation contained about 50% more amines than those of control rats. The plasma corticosterone concentrations in the nialamide-treated controls were within the normal range. When rats were immobilized for 6 hr during treatment with nialamide, the brain amine concentrations were increased to a similar extent as in the rats treated with nialamide alone. This, however, did not prevent the increase in plasma corticosterone caused by the restraint stress.

Blood pressure

Prenylamine, given subcutaneously in a dose of 100 mg/kg, as well as *p*-CPA, do not cause an appreciable change in arterial blood pressure. Prenylamine, however, given in a dose of 5 mg/kg intravenously, causes a profound fall of arterial

TABLE 1. *Effect of prenylamine, p-chlorophenylalanine (p-CPA) on brain amine and plasma corticosterone concentrations*

Treatment	Route of administration and dose	Hours after administration	5-HT	NA	DA	Plasma corticosterone
None (controls)	i.p. 0·9% NaCl	2	616±10 (50)	394± 4 (50)	492±21 (50)	20·8±2·4 (40)
Prenylamine	i.v. 5 mg/kg	2	465±10 (16)*	185±12 (16)*	305±26 (16)*	28·6±2·8 (10)*
		6	402±14 (16)*	248±15 (16)*	340±18 (16)*	12·0±1·5 (10)*
		24	610±21 (16)	330±12 (16)*	395±10 (16)*	19·2±3·7 (10)
	s.c. 100 mg/kg	6	480±26 (18)*	212±21 (18)*	281±21 (18)*	28·5±2·5 (22)*
		24	540±20 (12)*	301±18 (12)*	343±14 (12)*	15·7±2·9 (10)*
		168	601±15 (12)	531±10 (12)*	411± 8 (12)*	15·4±3·4 (10*
p-CPA	i.p. 316 mg/kg	6	660±29 (8)	340±15 (8)	440±20 (8)	18·6±2·4 (10)
		24	326±11 (12)*	303±11 (12)*	433±19 (12)*	25·4±2·9 (10)
		48	336±25 (12)*	295±14 (12)*	424±16 (12)*	23·0±2·2 (10)
		72	473±17 (8)*	297±11 (8)*	430±21 (8)*	20·6±3·1 (10)

5-HT, NA and DA values are expressed as ng/g of brain, plasma corticosterone concentrations in μg/100 ml. The values shown are means ± standard error of the mean. The figures within brackets show the number of estimations performed. * Significantly different from control values, $P<0·01$.

TABLE 2. *Effect of restraint stress in rats, pretreated or not with nialamide, on brain amine and plasma corticosterone levels*

Treatment	Hours after administration or restraint stress	5-HT	NA	DA	Plasma corticosterone
None (controls)		616±10 (50)	394± 4 (50)	492±21 (50)	20·8±2·4 (40)
Restraint stress (RS) (6 hr)		723±15 (16)*	327±10 (16)*	433±12 (16)*	43·3±4·5 (8)*
Nialamide	26	931±31 (16)**	640±15 (16)**	756±24 (16)**	19·2±1·8 (12)**
Nialamide + RS	26	1040±36 (18)**	621±25 (18)**	750±20 (18)**	49·3±3·1 (18)

5-HT, NA and DA values are expressed in ng/g, plasma corticosterone levels in μg/100 ml. The values shown are means ± standard errors. The figures within brackets show the number of determinations performed. * Significantly different from control values, $P<0·01$. ** Significantly different from animals undergoing restraint stress, $P<0·01$.

blood pressure which lasts from 5 to 7 min. This hypotension was also observed by Cession-Fossion (1967).

Discussion

The depletion of brain amine stores by reserpine, especially those of 5-HT, was considered to be the chief factor in the mechanism of the adrenocortical activation caused by this alkaloid (Westermann, Maickel & Brodie, 1962). The results obtained in the present experiments failed to demonstrate a direct correlation between the concentrations of monoamines in the brain and the release of ACTH, as indicated by the corticosterone concentrations in the plasma. The fall in the brain amine content 2 hr after an intravenous and 6 hr after a subcutaneous injection of prenylamine was accompanied by a rise in the plasma corticosterone concentration. During the following period of 22 hr, however, the brain amine concentration remained low whereas the plasma corticosterone had fallen to values below those observed in control rats. In addition, there was also no significant rise in plasma corticosterone in the rats in which the concentration of 5-HT in the brain was decreased after an intraperitoneal injection of *p*-chlorophenylalanine. Recently, Smelik (1967) has found that the hypothalamic depletion of monoamines is not essential for the ACTH-releasing effects of reserpine.

The increase in the brain amine concentration 26 hr after the administration of nialamide was not accompanied by a change in plasma corticosterone concentration. Animals in which substantial increases in plasma corticosterone concentrations occurred after immobilization showed a small increase in the concentration of 5-HT and a small decrease in the concentrations of NA and DA in the brain. Such changes in 5-HT and noradrenaline were reported by Rosencrans & de Feo (1965) to occur in a similar situation. The increased brain amine level after treatment with nialamide did not inhibit the rise in corticosterone secretion in restrained rats although an antagonism of the stress induced activation of the adrenal cortex by iproniazid, another MAO inhibitor, has been reported (Kothari, Saunders & Kline, 1961; Gaunt, Renzi & Chart, 1962). This effect has been contradicted by Pekkarinen, Tala, Niemela & Sotaniemi (1962), Manca, Miele, Passarelli & Preziosi (1963) and Ganong, Wise, Schackleford, Borycza & Zipf (1965). Lorenzen & Ganong (1967) have suggested that the ability of α-ethyltryptamine (another MAO inhibiting drug) to inhibit stress-induced adrenal cortical activation is not related to changes in the concentration of monoamines in the hypothalamus.

One cannot exclude, however, that the ratio between the different brain monoamines as well as their rates of synthesis, uptake and/or release may play a part in the mechanisms which control the release of hypothalamic neurotransmitters and possibly that of CRF. A chemically induced selective depletion of brain monoamines may provoke a different pattern of the oestrus cycle (Airaksinen & McIsaac, 1968). Studies, recently carried out by Lippmann (1968) in the hamster, have shown, indeed, that the relative availability of the hypothalamic monoamines is more important than their absolute concentrations with respect to gonadotrophin secretion.

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