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THE EFFECTS OF HYDRAZINES ON RAT BRAIN 5-HYDROXYTRYPTAMINE, NOREPINEPHRINE, AND GAMMA-AMINOBUTYRIC ACID*

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Abstract—The influence of hydrazine (HY), methylhydrazine (MMH), 1,1-dimethylhydrazine (UDMH), and 1,2-dimethylhydrazine (SDMH) was studied upon possible transmitter substances in rat brain, at times calculated just to precede convulsions. Mesencephalon–diencephalon, medulla, cortex, and cerebellum were separately examined.

All the hydrazines increased 5-hydroxytryptamine, particularly in cortex, where a three-fold increase was found.

All the hydrazines increased norepinephrine by small amounts (48% maximum), the effects being greatest in cortex and least in mesencephalon–diencephalon.

MMH, UDMH, and SDMH lowered γ-aminobutyric acid (GABA) by small amounts (17% maximum) in medulla, cortex, and mesencephalon–diencephalon, but effects on cerebellum were small or absent. HY raised GABA in all parts, especially in mesencephalon–diencephalon (31%).

Since the 'nontoxic' SDMH showed all of the above effects to the same extent as the toxic hydrazines, it is unlikely that these effects are causally involved in toxicity of these agents. An exception may be the effect of HY in raising GABA levels.

THE MODE of toxic action of hydrazine has been investigated for over 50 years,[1] and the recent use of alkylhydrazines as rocket fuels has reawakened interest in these compounds. Two possible mechanisms have been discussed. One is an interference with glucose metabolism, since hydrazine and alkylhydrazines give rise in rats to to hyperglycemia initially,[2] and hydrazine causes profound hypoglycemia after about a day in dogs and rabbits.[1] A second possibility is that hydrazines interfere with vitamin B_6, and hence with enzymes which require B_6, particularly with glutamic decarboxylase.[3–5]

The present studies explore in detail the possibilities of interference with levels in brain of potential transmitter substances whose levels may be controlled by B_6-requiring enzymes. These substances are: γ-aminobutyric acid (GABA), which is synthesized by glutamic decarboxylase; and 5-hydroxytryptamine (5HT) and norepinephrine (NE), both of whose biosyntheses involve aromatic L-amino acid decarboxylase.[6, 7]

The hydrazines used were hydrazine itself (HY), 1, 1-dimethylhydrazine (unsymmetrical dimethylhydrazine, UDMH), monomethylhydrazine (MMH) and 1, 2-dimethylhydrazine (symmetrical dimethylhydrazine, SDMH).

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MATERIALS AND METHODS

Female albino rats weighing 180 to 200 g were obtained from the Holtzman Co., Madison, Wis. HY and UDMH (free base forms) were obtained from Eastman Organic Chemicals, Rochester, N.Y.; and the free base of MMH and the dihydrochloride of SDMH from Chemicals Procurement Laboratories, New York. Fluorescent measurements were made with the Aminco–Bowman spectrophotofluorometer.

The free forms of HY, MMH, UDMH, and SDMH dihydrochloride were dissolved in saline, and the pH was adjusted to neutral with 10 N HCl or 10 N NaCH, and the volume adjusted. The doses (LD_{50}) were injected intraperitoneally into rats, which were decapitated after 75 min in the case of HY, UDMH, and SDMH and 35 min for MMH. The (LD_{50}) (mg/kg) were: HY, 74; MMH, 28; UDMH, 102.[2] An arbitrary high dose of 500 mg SDMH/kg was used. This dose gave no convulsions within 24 hr. Brains were removed as quickly as possible and divided as follows: the cerebellum was removed, then the medulla oblongata, and finally the central portion (mesencephalon–diencephalon) was cut away from the cerebral cortex. The time of sacrifice chosen was the average time immediately before the first convulsion, as determined in previous work.[2] Rats which convulsed sooner were discarded. By this procedure we were assured that only preconvulsant effects were examined.

Determination of 5HT

Several changes were made in the 5HT assay method described by Kuntzman *et al.*[8] About 600 mg of brain pooled from three rats was weighed and homogenized in 2 ml of 0·1 N HCl in a glass Potter–Elvehjem homogenizer which was cooled by ice water. The volume was made up to 5 ml by adding water and the homogenate mixed well. Two ml were pipetted into a 50-ml test tube, 0·2 ml of 2 N Na_2CO_3 solution was added to adjust the pH to about 10, and 1 ml of borate buffer (pH 10·0), about 3 g of NaCl, and 15 ml of butanol were added. The test tube was stoppered with a cork which was covered with Saran Wrap and shaken for 45 min on a wrist-action shaker. The tubes were centrifuged, and 10 ml of the butanol phase were transferred to another 50-ml test tube, 15 ml of heptane and 1·2 ml of 0·1 N HCl were added, and the tube was shaken for 10 min on the shaker and centrifuged again. One ml of aqueous phase was pipetted out, and 0·3 ml of concentrated HCl was added. Fluorescence was measured at 540 mμ, with activation at 300 mμ, 20 to 50 min after adding concentrated HCl. Recovery of 5HT added to brain homogenate was 90 to 115%.

Determination of NE

Minor changes were made in the NE assay method described by Kuntzman *et al.*[8] About 600 mg of brain pooled from three rats was weighed and homogenized in 5 ml of 0·01 N HCl in a glass Potter–Elvehjem homogenizer which was cooled by ice water. A 1·2-ml sample of homogenate was transferred to a 50-ml test tube, 3 g of NaCl and 13 ml of butanol were added, and the tube shaken for 1 hr on the shaker. After centrifuging, 10 ml of the butanol phase was transferred to another 50-ml test tube, 20 ml of heptane and 1·6 ml of 0·01 N HCl were added, and the tube was shaken for 15 min on the shaker and centrifuged. One ml of aqueous phase was pipetted out, and Kuntzman's method was followed, except that two times the sample volume and reagents, and a standard 1 × 1 cm cuvet were used. Calculation of NE was based on internal standard which applied for each run. The recovery of added NE from brain homogenate was 88 to 117%.

The effect of pyridoxal phosphate hydrazone (produced endogenously during poisoning) on the NE determination was tested as follows. After the same procedure as above, the fluorescence intensity of the usual 1·2 ml of brain homogenate from a poisoned rat (A) was compared with that of a mixture of 0·6 ml of homogenate plus 0·6 ml of 0·01 N HCl containing 0·257 μg of NE (B), and with that of 1·2 ml of the acid containing 0·257 μg of NE alone (C). The effect of the hydrazone was measured by comparing the fluorescence values for (B−A/2) with that for C. In the absence of interference, B−A/2 = C. This procedure was followed for all four hydrazines.

Determination of GABA

The most specific methods available for GABA are the enzymatic method of Jakoby and Scott[9] and the chromatographic method of Maynert and Kaji.[3] The large number (180) of determinations required in the present study made the fluorometric method of Lowe *et al.*[10] attractive. To confirm that the modified Lowe method was specific, GABA was determined in rat brain cortex by Lowe's method and independently by Maynert's method; the values found were respectively 0·46 mg/g ± 0·44 (SEM) and 0·37 mg/g ± 0·020 (SEM). Values for GABA (mg/g of whole rat brain) in the literature are 0·40 (Medina 1963 [11]), 0·29 (Maynert and Kaji 1962 [3]), 0·21 (Ansell and Richter 1954 [12]), 0·29 (Cravioto *et al.* 1951 [13]), and 0·63 (Awapara *et al.* 1950 [14]). The value for whole brain by the modified Lowe method (calculated from data of Table 4 along with the per cent weight contribution of each part) is 0·46. It was concluded that the modified Lowe method was suitable to demonstrate any substantial changes in GABA levels. Lowe's method[9] was adapted for semimicro scale, with minor changes. The same brain homogenates were used as for NE determination. An equal volume of 10% trichloracetic acid was added to 1·0 ml of brain homogenate in 0·01 N NCl and centrifuged; 0·1 ml of supernatant was pipetted into 10-ml test tubes and 0·015 ml of 0·05 M glutamic acid in 0·2 M phosphate buffer (pH 6·4) and 0·2 ml of the ninhydrin solution were added. The tubes were kept at 60° for 30 min, cooled, and 5·0 ml of the copper tartrate reagent solution added. After 15 min, fluorescence was measured at 450 mμ, activating at 380 mμ.

RESULTS

Effects on 5HT

The distribution of 5HT in rat brain is shown in Table 1. Mesencephalon–diencephalon contained more 5HT than the other parts, and cerebellum was the poorest source of 5HT. Table 1 also indicates that all hydrazines tested increased the 5HT level of cortex very substantially (two- or three-fold), with lesser increases in other brain parts. SDMH increased 5HT more than the other hydrazines.

Effects on NE

The distribution of NE in rat brain was determined. Mesencephalon–diencephalon contained more NE than the other parts, and cerebellum showed the lowest level (Table 2). All the hydrazines tested, including the 'non-toxic' SDMH, had a tendency to increase the NE level, although the increases were small (maximum 42%) and significant only in six cases.

There was a possibility that the various hydrazones of pyridoxal phosphate might interfere with the NE determination (A. A. Wykes, personal communication). Medina[15] demonstrated that hydrazones are formed in brain when rats are given

UDMH along with PAL or PALP. However, NE added to the brain homogenate from poisoned and normal rats was recovered 88 to 117%, as shown in Table 3 (see procedure under Methods). Furthermore SDMH, which should not react to form hydrazones, also gave the same recovery as the other hydrazines under our conditions. The hydrazones thus do not interfere with the fluorometric NE determination.

TABLE 1. 5-HYDROXYTRYPTAMINE LEVELS IN BRAINS OF RATS TREATED WITH HYDRAZINES AT LD_{50}

Compound injected	Mesencephalon–diencephalon	Medulla oblongata	Cerebral cortex	Cerebellum
	(μg 5HT/g brain[1]*)			
None	1·08 ± 0·15	0·69 ± 0·10	0·16 ± 0·01	< 0·01
HY	1·40† ± 0·13	0·95† ± 0·08	0·44† ± 0·03	< 0·01
MMH	1·24 ± 0·10	0·88 ± 0·14	0·45† ± 0·004	< 0·01
UDMH	1·02 ± 0·16	0·70 ± 0·13	0·39† ± 0·07	< 0·01
SDMH	1·70† ± 0·09	1·11 ± 0·14	0·52† ± 0·06	0·07† ± 0·06

* Figures are mean values with standard errors. The mean values were obtained from triplicate determinations on each of three experiments with three pooled brains.

† These values are significantly different from controls as judged by the "t" test at the 5% level.

TABLE 2. NOREPINEPHRINE LEVEL IN BRAINS OF RATS TREATED WITH HYDRAZINES AT LD_{50}

Compound injected	Mesencephalon–diencephalon	Medulla oblongata	Cerebral cortex	Cerebellum
	(μg NE/g brain*)			
None	0·86 ± 0·089	0·50 ± 0·051	0·46 ± 0·028	0·26 ± 0·048
HY	0·88 ± 0·129	0·60 ± 0·097	0·57† ± 0·108	0·30 ± 0·091
MMH	0·86 ± 0·135	0·61† ± 0·055	0·50 ± 0·062	0·28 ± 0·012
UDMH	1·00 ± 0·026	0·58 ± 0·034	0·68† ± 0·049	0·037† ± 0·039
SDMH	0·84 ± 0·107	0·61† ± 0·015	0·45 ± 0·034	0·32† ± 0·043

* See footnotes to Table 1.

TABLE 3. RECOVERY OF NOREPINEPHRINE FROM BRAIN OF NORMAL AND POISONED RATS

Compound injected	Recovery	Average
None	103, 89	96
HY	102, 104	103
MMH	117, 111	114
UDMH	98, 117	108
SDMH	88, 108	98

Effects on GABA

The distribution of GABA in rat brain is shown in Table 4. The GABA levels tended to be lowered by the nontoxic SDMH as well as by the toxic MMH and UDMH, but was raised by HY; however, these effects were small (e.g. a 31% increase by hydrazine) and were significant in only five cases.

DISCUSSION

The findings of substantial increases in 5HT, and lesser increases in NE, suggest that rather than interfering with their synthesis via the B_6-requiring aromatic L-amino acid decarboxylase, the prime effect is upon their destruction, presumably by monoamine oxidase, which is not a B_6-requiring enzyme. Recently it has been shown by a manometric procedure with 5HT as substrate that SDMH, UDMH, and MMH are quite good inhibitors of rat brain monoamine oxidase *in vivo*; but HY is ineffective (A. A. Wykes, personal communication). However, in our studies, HY is about as effective as the others in raising 5HT levels in cortex.

TABLE 4. γ-AMINOBUTYRIC ACID LEVEL IN BRAIN FROM RATS TREATED WITH HYDRAZINES AT LD_{50}
(mg GABA/g brain)

Compound injected	Mesencephalon-diencephalon	Medulla oblongata	Cerebral cortex	Cerebellum
None	0·54 ± 0·067	0·40 ± 0·042	0·46 ± 0·044	0·36 ± 0·024
HY	0·71† ± 0·050	0·46 ± 0·012	0·53 ± 0·066	0·46† ± 0·042
MMH	0·53 ± 0·005	0·34 ± 0·007	0·40 ± 0·029	0·34 ± 0·048
UDMH	0·45† ± 0·015	0·34 ± 0·012	0·41† ± 0·017	0·36 ± 0·021
SDMH	0·48 ± 0·029	0·35 ± 0·006	0·41† ± 0·017	0·36 ± 0·080

*† See footnotes to Table 1.

The increase in 5HT is not in accord with the possibility raised by Bonnycastle *et al.*[16] that increases in brain 5HT might have an anticonvulsant action.

All the effects of HY, MMH, and UDMH were produced also by the nontoxic SDMH, and this is evidence against a causal connection between the toxicity of these agents and levels of 5HT, NE, or GABA. Medina[10] also concluded from studies of whole rat brain that GABA levels were not important, since (a) pyridoxine could prevent convulsions by UDMH and MMH, yet restored the GABA level fully only with MMH; (b) pyridoxal potentiated the convulsant actions of UDMH and MMH, with negligible change in GABA levels; and (c) SDMH was as effective as MMH or UDMH in lowering GABA (in harmony with our above observations) yet is 'nontoxic'.

In spite of these anomalies, we feel that the role of GABA in poisoning cannot yet be dismissed, in spite of the views of Medina[11] and Maynert and Kaji.[3] It has been pointed out in our previous work that HY is quite unlike MMH and UDMH in that at LD_{50} it is not a convulsant but a depressant,[2] and this correlates with the fact that HY raises brain GABA in whole brain of rats[11] and mice[3] and in all parts of rat brain (Table 4), whereas MMH and UDMH lower it. Furthermore, pyridoxal profoundly lowers the GABA level in HY-poisoned rats[11] and is weakly therapeutic in HY poisoning, whereas it weakly synergizes UDMH and MMH poisoning.[2]

Two methodological points deserve consideration. We have insisted on studying preconvulsant phenomena, whereas other workers have routinely taken rats after their first convulsion. Our view is that we wish to know the causes rather than the effects of the convulsions. To do this we determined for each compound the average time to the first convulsion, and in later work took all rats at this predetermined time and discarded those that had convulsed. This had the disadvantage that it requires careful prior studies of dose–effect responses; is uneconomical in rats, since about

half of them have to be discarded; and it involves a biased sample of rats, since the more sensitive ones are discarded. Nevertheless, we feel our data tell us more about the prerequisites for convulsions.

We have also routinely examined four portions of brain. This is inconvenient in an animal as small as the rat, and quadruples the effort. However, fairly important differences were found: e.g. MMH and SDMH increase NE in the medulla about 20%, without any effect on that in the mesencephalon–diencephalon. MMH increases the 5HT in the cortex by 181% but in the mesencephalon–diencephalon only by 15%, in spite of (perhaps because of) the fact that the former is normally sevenfold richer in 5HT than is the latter.

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