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Original Investigations

Monoamines in the Brain under the Influence of Muscimol and Ibotenic Acid, Two Psychoactive Principles of Amanita Muscaria

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Abstract. The concentrations of noradrenaline, dopamine and serotonin were measured in the brain of male albino mice and rats after intraperitoneal injections of muscimol, ibotenic acid or LSD. All three drugs induced a generalized increase of serotonin. When muscimol was administered to rats after pretreatment with p-chlorophenylalanine, a serotonin synthesis inhibitor, the serotonin concentration was still increased in midbrain and hypothalamus. Muscimol also caused a reduced accumulation of 5-hydroxyindoleacetic acid in rats pretreated with probenecid. There were differences in the action of the three compounds on the catecholamine concentration. Muscimol and LSD caused a decrease of the catecholamines. Ibotenic acid increased the catecholamine concentration. Certain topographical differences were noted.

The increase in the serotonin concentration in the hypothalamus and midbrain after muscimol may be due to a reduced turnover of serotonin. An increase in serotonin concentration and a decrease of 5-hydroxyindolacetic acid in the rat brain are effects observed also with other psychotomimetic drugs such as LSD or psilocybin.

Key-Words: Muscimol, Ibotenic Acid, LSD — Monoamine Concentrations in Brain — 5-Hydroxyindoleacetic Acid — p-Chlorophenylalanine — Probenecid — Turnover of Serotonin.

Introduction

Some hallucinogens such as Lysergid (LSD) habe been found to cause an increase in serotonin concentration and a decrease in catecholamine concentration in the rat brain (Woolley *et al.*, 1954; Freedman, 1961; Rosecrans *et al.*, 1967).

Two years ago, muscimol, ibotenic acid (Fig. 1) and related compounds were isolated from amanita muscaria (Eugster and Takemoto, 1967). Waser (1967) reported some pharmacological and psychotomimetic effects of these two drugs. In mice, their injection produced sympathetic effects which at least in part may be of central origin. The central control

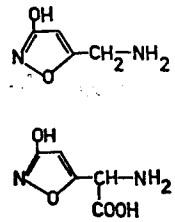

Fig. 1. Molecular structure of muscimol and ibotenic acid. muscimol = 5-aminomethyl-3-hydroxy-isoxazol, ibotenic acid = α-amino-3-hydroxy-5-isoxazol-acetic acid

of motor activity is also impaired with the development in mice of ataxia, catalepsy, convulsions and muscle twitches. With higher doses all treated animals showed sedation.

We investigated the influence of these substances on brain monoamines. Measurements of the monoamine concentrations in the whole brain of mice furnished only general information. More details were obtained by measuring the concentration changes in different regions of the rat brains. In order to gain some insight into the turnover of serotonin, we studied its concentration after administration of a synthesis inhibitor and the concentration of its metabolite, 5-hydroxy-indoleacetic acid (5-HIAA).

Methods

Animals. Male albino mice (20—25 g body weight) of a random bred ICR COBS (cesarian originated barrier sustained) strain from Charles River Mouse Farms, and male albino rats (180—220 g body weight) of a random bred CF (Carworth Farms) strain were used. The animals were provided by the Animal Breeding Institute of the Department of Veterinary Medicine, University of Zürich.

Drugs. The following drugs, dissolved in saline, were administered in total volumes of 0.1 ml/100 g:

muscimol	3 mg/kg i.p.
ibotenic acid	16 mg/kg i.p.
LSD	10 mg/kg i.p.
p-chlorophenylalanine	300 mg/kg i.p.
probenecid	300 mg/kg p.o.
0.9% NaCl	1 ml/kg i.p. (control animals)

Brain Sectioning. After decapitation the rat brains were quickly removed and cut just behind the medulla oblongata. The cerebellum,

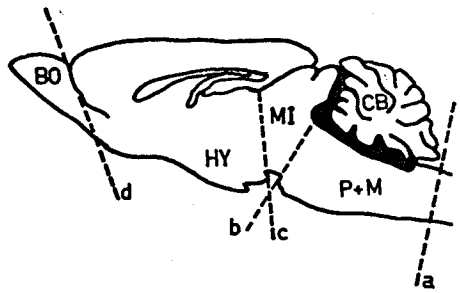

Fig. 2. Sagittal section of rat brain (König and Klippel, 1963). The dotted lines (*a, b, c, d*) represent frontal cuttings. *BO* bulbus olfactorius; *HY* hypothalamus with median thalamus and medial hippocampus; *MI* midbrain; *P + M* pons and medulla oblongata; *CB* cerebellum

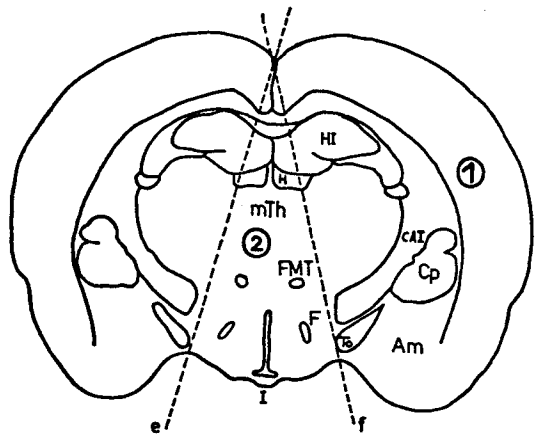

Fig. 3. Frontal section of rat brain (König and Klippel, 1963). The dotted lines represent sagittal cuttings (*e, f*). *HI* hippocampus; *H* habenula; *mTH* median thalamus; *FMG* fasciculus mamillothalamicus; *F* columna fornicis; *I* infundibulum; *CAI* capsula interna; *Cp* nucleus caudatus putamen; *Am* amygdala; *To* tractus opticus. 1. "Forebrain" contains cortex, striatum, amygdala and lateral parts of thalamus. 2. "Hypothalamus" contains hypothalamus, median thalamus and medial hippocampus

the olfactory bulb and the pineal gland were discarded. Amine determinations were performed in four brain areas: Medulla oblongata + pons, midbrain, hypothalamus + median thalamus, remaining forebrain. The position of the various cuts is given in Figs. 2 and 3.

Fluorimetric Determination. Noradrenaline was determined according to Bertler *et al.* (1958), dopamine according to Carlsson and Waldeck (1958) and 5-hydroxytryptamine according to Udenfriend *et al.* (1958).

Table 1. *Monoamine concentrations (ng/g fresh tissue) in the mouse brain two hours after the i.p. injection of 16 mg/kg ibotenic acid, 3 mg/kg muscimol or 10 mg/kg LSD*

		Ibotenic acid	Muscimol	LSD
		±S.D. (*n*)	±S.D. (*n*)	±S.D. (*n*)
NA	*T*	539±80 (5)	447±44 (3)	249± 76 (5)
	C	485±55 (3)	474±47 (3)	269± 76 (7)
DA	*T*	426±30 (4)	432±51 (3)	519±172 (7)
	C	354±37 (4)	367±34 (3)	603±100 (7)
		$p \leq$ 0,0025	$p \leq$ 0.05	
5-HT	*T*	363±77 (11)	399±83 (8)	342± 15 (3)
	C	368±81 (8)	360±91 (6)	300± 25 (3)
			$p \leq$ 0.10	$p \leq$ 0.05

Abbreviations for Table 1—4.

S.D.	standard deviation
(*n*)	number of experiments each with 4—5 animals
T	mean concentration in the test brain tissue
C	mean concentration in the control brain tissue
p	confidence coefficient
FB	forebrain
HY	hypothalamus
MI	midbrain
P + M	pons and medulla oblongata.

with slight modifications (Häggendal, 1962). We added 0.1 ml of 2% ascorbic acid and 0,2 ml of 10% EDTA to 10 ml aqueous solution. 5-HIAA was estimated fluorimetrically as described by Roos (1962) and Sharman and Smith (1962). In the experiments on mice, 4 whole brains were pooled, whereas in the studies on rats 5 brain sections were used for one determination. Care was taken to start the experiments always at the same time of the day. The average recovery of the monoamines which were added to tissue homogenate and carried through the procedures ranged between 85 and 95 percent. The values in the tables are corrected for this recovery.

Statistical Analysis. Differences of the fluorescence intensity between test and control samples were analysed by means of the *F*-test and *t*-test comparison (Doerffel, 1965). Differences between the means of treated and control animals are considered to be significant if the confidence coefficient is 95% ($p < 0.05$) or more.

Results

There is some similarity between the effects of muscimol and LSD on the overall monoamine concentration in the mouse brain (Table 1).

Table 2. *Monoamine concentrations in four different regions of rat brain (ng/g fresh tissue) two hours after the injection of ibotenic acid (16 mg/kg i.p.)*

		FB	*HY*	*MI*	*P + M*
		±S.D. (*n*)	±S.D. (*n*)	±S.D. (*n*)	±S.D. (*n*)
NA	*T*	195± 84 (4)	752±306 (4)	1056±428 (4)	513±179 (4)
	C	229+102 (8)	531+237 (6)	542+161 (7)	404+110 (6)
		$p \leq$ 0.10		$p \leq$ 0.01	
DA	*T*	366± 77 (4)	768±209 (4)	465± 43 (4)	390±245 (4)
	C	384± 86 (5)	574±146 (5)	398±141 (6)	177± 30 (5)
			$p \leq$ 0.10		$p \leq$ 0.05
5-HT	*T*	294± 68 (5)	721± 82 (5)	804± 47 (4)	454± 87 (5)
	C	259± 70 (7)	612±117 (9)	685±101 (9)	413± 83 (9)
			$p \leq$ 0.05	$p \leq$ 0.025	$p \leq$ 0.10

Table 3. *Monoamine concentrations in four different regions of rat brain (ng/g fresh tissue) two hours after the injection of muscimol (3 mg/kg i.p.)*

		FB	*HY*	*MI*	*P + M*
		±S.D. (*n*)	±S.D. (*n*)	±S.D. (*n*)	±S.D. (*n*)
NA	*T*	200± 49 (5)	645±250 (5)	392±205 (5)	389±122 (5)
	C	241± 44 (4)	765±315 (5)	430± 81 (5)	554±103 (5)
					$p \leq$ 0.05
DA	*T*	548±209 (5)	961±234 (4)	552±389 (5)	393±104 (4)
	C	809±179 (5)	1245±339 (4)	772±334 (4)	326±106 (4)
		$p \leq$ 0.05			
5-HT	*T*	356± 85 (5)	819±321 (4)	808±128(5)	560±196 (5)
	C	275+ 43 (6)	670+248 (4)	706+119 (5)	464+139 (5)
		$p \leq$ 0.05			

The serotonin concentration increased and the noradrenaline concentration decreased. However the dopamine concentration increased after muscimol in contrast to a decrease after LSD.

After ibotenic acid an increase was observed in noradrenaline and dopamine concentrations in the mouse brain, whereas the serotonin concentration did not change. The relatively small changes in monoamine concentration in the mouse brain could originate from large changes in small brain areas. Therefore, the influence of muscimol, ibotenic acid and LSD was investigated in four different regions of the rat brain (Tables 2 and 3).

Following the injection of ibotenic acid the noradrenaline content was markedly increased in the hypothalamus, midbrain and pons

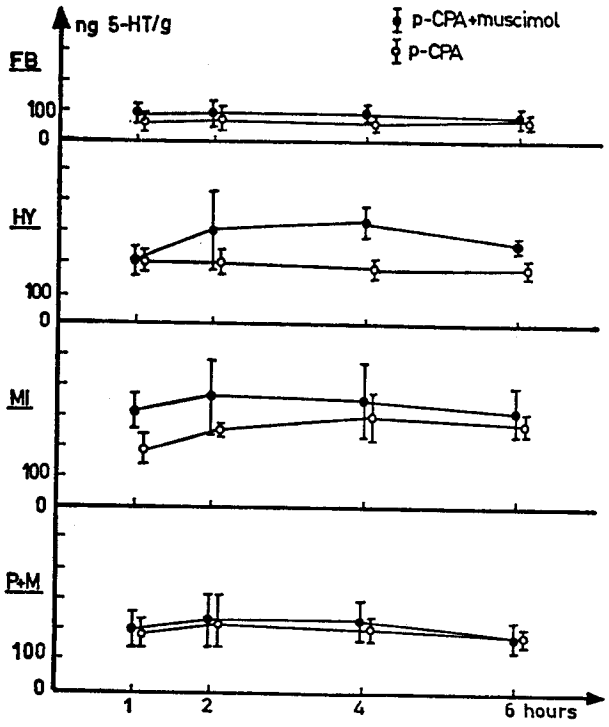

Fig. 4. Serotonin concentration in rat brain one, two, four and six hours after injection of muscimol (3 mg/kg i.p.). The animals were pretreated with p-chlorophenylalanine (300 mg/kg i.p.) 48 hours before the muscimol injection. *FB* forebrain; *MI* midbrain; *HY* hypothalamus; *P + M* pons and medulla oblongata

+ medulla oblongata (Table 2). The dopamine concentration was increased in the hypothalamus and pons + medulla oblongata, and showed a tendency to rise in the midbrain and to fall in the forebrain. Serotonin concentrations increased especially in hypothalamus and midbrain.

After the injection of muscimol the noradrenaline concentration was markedly lowered in pons and medulla oblongata (Table 3), and a similar tendency was found in all other regions. Dopamine decreased markedly in the forebrain. A slight decrease was also observed in the hypothalamus and midbrain, with an increase in pons and medulla oblongata. Serotonin levels were distinctly increased in all parts, but the change was only significant in the forebrain.

The generalized increase of serotonin induced by muscimol, ibotenic acid was well as by LSD suggested further investigations. 48 hours after administration of p-chlorophenylalanine, which inhibits the 5-HT syn-

Table 4. *5-hydroxy-indole-acetic acid concentration (ng/g fresh tissue) in four different regions of rat brain one hour after the injection of muscimol (3 mg/kg i.p.). The animals were pretreated with probenecid (300 mg/kg p.o.) for two hours*

		FB	*HY*	*MI*	*P + M*
		±S.D. (*n*)	±S.D. (*n*)	±S.D. (*n*)	±S.D.(*n*)
5-HIAA	*T*	586±227 (5)	677±128 (6)	1305±222 (6)	857± 91 (6)
	C	601±220 (6)	931±216 (5)	1625±377 (6)	1283±364 (5)
			$p \leq$ 0.025	$p \leq$ 0.05	$p \leq$ 0.01

thesis by blocking the tryptophan hydroxylase, the serotonin concentration was markedly reduced to 20% of the normal value in all brain regions (Fig. 4). The value of the control group showed slight changes which could be due to diurnal variations. After this pretreatment, muscimol increased the serotonin concentration in the midbrain and hypothalamus. The effect of muscimol was noticed first in the midbrain and lasted 6 hours in the hypothalamus (Fig. 4).

The measurement of 5-HIAA, the acid metabolite of 5-HT, was expected to yield information about the turnover of 5-HT. Pretreatment with probenecid blocks the permeation of 5-HIAA through the blood-brain-barrier (Neff and Tozer, 1968) and thus amplifies certain differences in the 5-HIAA concentration. Two hours after pretreatment with probenecid there was a significant decrease of 5-HIAA induced by muscimol (Table 4).

Discussion

All three drugs investigated increased the serotonin concentration in the brain, the effects differing only quantitatively from each other. The findings after LSD are in agreement with earlier investigations (Daniel and Freedman, 1963; Rosecrans *et al.*, 1967).

When the synthesis of 5-HT was blocked by p-chlorophenylalanine, muscimol still increased the serotonin concentration in hypothalamus and midbrain, but it lowered the formation of 5-HIAA. The increase in serotonin concentration brought about by muscimol may be due in part to a decreased release and/or metabolism of 5-HT. Inhibition of monoamine oxidase is unlikely considering the observed symptoms (sedation and hypothermic effects) and the decrease in catecholamine concentrations. The decreased turnover rate could rather result from a diminished 5-HT release by neurons due to a decrease of the impulse flow in the serotoninergic neurons (Andén *et al.*, 1968; Corrodi and Malmfors, 1966).

5-HT concentration was affected by muscimol and ibotenic acid to about the same degree in all brain regions. After inhibition of 5-HT syn-

thesis forebrain, pons and medulla no longer showed an increase of 5-HT after muscimol. The elevated hypothalamic concentration, when considered together with the low 5-HIAA formation, suggested a reduced release of 5-HT. Besides this, a change in the rate of synthesis might have played a role in the increase in 5-HT concentration observed without synthesis inhibition. It is interesting to note that after synthesis inhibition muscimol produced initially a rise of 5-HT concentration in the midbrain which contains the bulk of the nerve cell bodies (Dahlström and Fuxe, 1964), and this was later reduced to control values. At the time of maximal sedation (2—4 hours after injection of muscimol) the change was more pronounced in the nerve terminal region.

There are certain differences between the action of the three compounds investigated on the catecholamine concentrations: muscimol and LSD were similar in their effect on the monoamine concentration in the rat brain, both inducing a decrease in catecholamines and an increase in serotonin. The same is true for noradrenaline and serotonin in the mouse brain, whereas in this species the two drugs showed different effects on the dopamine concentration. The reason for the difference between the increase in dopamine concentration after muscimol in the mouse brain and the decrease in the rat brain is unknown.

In contrast to these two drugs ibotenic acid increased the catecholamine concentration. Certain topographical differences in the magnitude of the change in NA and DA concentrations were noted (Tables 2 and 3), but they were rather irregular and do not permit many conclusions to be drawn on the topography of CA-containing systems. The fact that after ibotenic acid dopamine concentration remained practically unchanged in the forebrain (with basal ganglia) and in the midbrain, might indicate that its concentration in the nigro-neostriatal system did not alter and thus, that the changes observed especially in pons and medulla might result from its occurence as a precursor of NA. In contrast, muscimol markedly reduced the DA concentration in the forebrain and the midbrain, which suggests that besides NA neurons it also affected DA neurons. The significance of the changes in the catecholamine concentrations with respect to turnover changes has not yet been studied.

It does not seem possible at the present time to establish a close correlation between the functional effects of these drugs and their biochemical actions. Substances with LSD-like behavioral effects, such as psilocybin, mescalin or l-acetyl-LSD produce an increase in brain 5-HT concentration and a decrease in catecholamine concentration (Freedman, 1963; Giarman and Freedman, 1965; Aghajanian and Freedman, 1967). Rosencrans *et al.* (1967) found that after the injection of LSD (200 µg/kg i.v.) into rats the 5-HT concentration increased and at the same time the

5-HIAA concentration decreased in the rat brain. This could be due to a diminished release of 5-HT.

Since muscimol and, to a lesser extent, ibotenic acid possess certain psychotomimetic properties (Waser, 1967; Theobald *et al.*, 1968), it is tempting to look for correlations between such symptoms and certain similarities in the biochemical effects, notably that on serotonin. One common action of all three drugs was possibly to lower the impulse flow in the serotoninergic neurons. It remains to be elucidated whether this was due to a stimulation of serotonin receptors with consequent negative feedback action on the respective neurons, as suggested by Andén *et al.* (1967, 1968) and Corrodi *et al.* (1967), or to a transsynaptically induced reduction of activity in these neurons.

However, the biochemical effects need not be correlated exclusively to behavioral changes. They may also reflect an involvement of neurons of other functional systems, e.g. central representations of vegetative functions. With regard to the changes in DA concentration in the mouse brain, the similarity of the actions of both muscimol and ibotenic acid on the dopamine concentration in this species, and the fact that both drugs caused extrapyramidal symptoms is most interesting.

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