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Brain Monoamine Oxidase Activity After In Vivo-Chronic Iprindole Treatment

BERTHA G. ORTEGA-CORONA, JOSÉ CARRANZA,[1] AGUSTÍN SOSA, PATRICIA GUZMÁN-AMAYA, NORA S. ESPARZA-AVALOS AND GUADALUPE CASTRO-OSUNA

Unidad de Investigación Biomédica del Centro Médico Nacional
División de Biología Molecular, Sección de Neuroquímica
Apartado Postal 73-032, México 73, DF México

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ORTEGA-CORONA, B. G., J. CARRANZA, A. SOSA, P. GUZMÁN-AMAYA, N. S. ESPARZA-AVALOS AND G. CASTRO-OSUNA. *Brain monoamine oxidase activity after in vivo-chronic iprindole treatment.* PHARMAC. BIOCHEM. BEHAV. 15(6) 951–954, 1981.—The effects of chronic administration of three different doses of iprindole on the monoamine oxidase activity and neurotransmitter content were studied in the cerebral cortex, the mesencephalon and the cerebellum of mouse brain. The treatment inhibited mitochondrial monoamine oxidase activity of all three brain regions studied, although a dose-response inhibitory effect was found only in the mesencephalon. Brain regional serotonin contents were markedly increased after iprindole treatment. The 5-hydroxyindoleacetic acid contents showed a significant decrease only in the cerebral cortex. Neither dopamine nor norepinephrine brain regional contents were significantly altered. On the basis of these results it is suggested that a substrate-specific inhibition of monoamine oxidase is involved in the mechanism of action of this tricyclic antidepressant.

Iprindole Monoamine Oxidase Serotonin Norepinephrine Dopamine Inhibition *In vivo* studies Brain

IT has been assumed that tricyclic antidepressant drugs (TAD) mainly act by inhibiting the neuronal reuptake of biogenic amines [4, 7, 20]. Accumulating evidence indicates, however, that transmitter reuptake inhibition is just one of several mechanisms of the action of TAD [11, 16, 19, 26]. Studies with iprindole, an antidepressant agent more potent than imipramine [2, 8, 15, 18, 19], have provided suggestive evidence that this tricyclic compound may produce its effect not by reuptake inhibition but by inhibition of monoamine oxidase (MAO) (monoamine: O_2 oxidoreductase-deaminating-EC 1.43.4) [21, 23, 24]. Although experimental evidence obtained by acute treatment with iprindole failed to support this suggestion [21], it is important to notice that antidepressants generally require long time of action for showing their effects. This communication reports a study of brain MAO activity in mice treated with iprindole over a prolonged period of time, and brain monoamine levels observed.

METHOD

Charles River CD-1 strain male mice were housed, 10 in a cage, at a controlled temperature room at 24±1°C and constant humidity, with free access to food and water. The light cycle was set at 12 hrs dark and 12 hrs light.

Four groups of 32 animals each were treated daily with 0, 0.5, 1.0, 2.0 mg/kg of iprindole dissolved in 0.2 ml of 0.9% saline solution for 120 days. Each group was divided into four subgroups of 8 animals, which were used to determine either (a) MAO activity, (b) dopamine (DA), (c) norepinephrine (NE), or (d) 5-hydroxytryptamine (5-HT) and its catabolic product 5-hydroxyindoleacetic acid (5-HIAA).

The animals were killed by decapitation at 9:00 hrs. Brains were removed rapidly and chilled to 4°C. Each brain was dissected to obtain the entire cerebral cortex, mesencephalon and cerebellum according to the techniques described by Glowinski [15]. Each brain region was individually homogenized in appropriate solution either for isolation of the mitochondrial fraction or extraction of biogenic amines.

Mitochondrial fractions were essentially prepared by following the method of Achee *et al.* [1], except that treatment with nagarse was omitted. Since further purification was not always obtained by density gradient centrifugation, the last step of the technique described by Achee was omitted. In order to corroborate the effectiveness of the method, mitochondria were initially isolated from the whole brain. The purification course of the mitochondrial fractions was followed, during the isolation process, by measuring MAO activity [12]. In addition, the morphology of the isolated mitochondrial fractions was examined by electron microscopy. Accordingly, samples were fixed in cold medium consisting of 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4.

After the effectiveness of the Achee method had been

[1]To whom reprint requests should be sent. Present address: Department of Psychiatry, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030.

TABLE 1

MONOAMINE-OXIDASE ACTIVITY IN "WASHED" MITOCHONDRIAL FRACTION FROM DIFFERENT REGIONS OF IPRINDOLE TREATED MOUSE BRAIN

Dose of Iprindole mg/kg/day	Brain Structure: Cerebral Cortex	Mesencephalon	Cerebellum
0.5	242 ± 11†	619 ± 12*	1463 ± 39†
1.0	223 ± 12†	527 ± 14†	2015 ± 45*
2.0	224 ± 5†	452 ± 9†	1750 ± 73*
Control	402 ± 19	920 ± 27	2578 ± 62

MAO activity was determined using ^{14}C-tryptamine as substrate. Enzymic activity is expressed as nmoles of substrate metabolized per hr per mg of protein at 37°C. Each value represents the mean ± SEM of 8 determinations.

*$p<0.01$ compared to controls (t-test).

†$p<0.001$ compared to controls (t-test).

assured, MAO activity was routinely measured by the radiometric assay of Wurtman and Axelrod [28]. Monoamine levels were measured according to Welch and Welch [27], and protein concentration according to Lowry *et al.* [13] using bovine serum albumin as standard.

RESULTS

Electron microscopy examination of the mitochondrial fraction showed a great number of mitochondria, all with a better morphological integrity. As judged by the 4-fold increase in MAO specific activity, the brain mitochondrial fraction was substantially purified. These results are similar to those reported by Achee *et al.* [1].

Table 1 shows the mitochondrial MAO specific activities of cerebral cortex, mesencephalon and cerebellum as well as the effects of varying doses of iprindole upon these activities. MAO activity was measured in the presence of ^{14}C-tryptamine bisuccinate (side chain-2-^{14}C) as substrate. In control animals, the highest specific activity was observed for cerebellar MAO (2578±62 nmoles/hr/mg protein), followed by mesencephalic (920±27 nmoles/hr/mg protein), and cerebral cortical (402±19 nmoles/hr/mg protein) MAO. These brain regional differences were statistically significant ($p<0.001$).

In drug treated animals, the ability of iprindole to inhibit monoamine oxidase deamination of tryptamine was independent of the brain area examined and the dose of iprindole administered. In the mesencephalon the enzyme activity was progressively inhibited by increasing dosages of iprindole. However, while treatment with 0.5 mg of iprindole caused a 32% inhibition of the enzyme activity, a fourfold increase in the dosage of iprindole caused only a slight additional (to 50%). The enzyme activities in other parts of the brain examined were entirely independent of the dosage of iprindole given. A constant inhibition (43% of the MAO activity) was observed in the cerebral cortex, and in the cerebellum the highest inhibitory effect was achieved by the lowest dose of iprindole administered.

Table 2 shows the 5-HT and 5-HIAA levels of the cerebral cortex, the mesencephalon, and the cerebellum of animals treated chronically (120 days) with iprindole or saline. Brain regional 5-HIAA concentrations were found to be almost twice as high as the 5-HT levels, in the control animals. Of the various brain structures examined, the mesencephalon showed significantly lower values of 5-HT and 5-HIAA concentrations than cerebral cortex and cerebellum. These two latter brain regions did not differ significantly.

The brain 5-HT content was markedly increased after iprindole treatment. The greatest 5-HT increase was observed in the cerebral cortex where it increased to 224 per cent of control values. The increase of the 5-HT levels was not related to the amount of iprindole administered in either the cerebral cortex or in the cerebellum. However, mesencephalic 5-HT content showed a steady increase related to increasing dosages of iprindole administered. 5-HIAA levels decreased significantly in the cerebral cortex after iprindole treatment. Cerebellar 5-HIAA contents were not modified by this treatment and mesencephalic levels decreased significantly only when the largest dose of iprindole was used. 5-HT levels showed a 4-fold variation compared to 5-HIAA levels, independent of the brain region studied.

Table 3 indicates DA and NE levels of the three brain regions studied. The DA values of the cerebral cortex and the mesencephalon were similar, while those of cerebellum were significantly higher. The NE values showed a very specific brain regional distribution. The relative values of NE were highest for the mesencephalon, intermediate for the cerebral cortex and lowest for the cerebellum. Surprisingly,

TABLE 2

EFFECT OF IPRINDOLE ON THE LEVELS OF 5-HYDROXYTRYPTAMINE AND 5- HYDROXYINDOLEACETIC ACID IN DIFFERENT REGIONS OF MOUSE BRAIN

Dose of Iprindole	Brain Structure: Cerebral Cortex 5-HT	Cerebral Cortex 5-HIAA	Mesencephalon 5-HT	Mesencephalon 5-HIAA	Cerebellum 5-HT	Cerebellum 5-HIAA
0.5	467 ± 9*	289 ± 12*	247 ± 11*	286 ± 17	372 ± 9*	418 ± 20
1.0	450 ± 15*	269 ± 10*	268 ± 9*	265 ± 17	323 ± 10*	370 ± 18
2.0	443 ± 13*	265 ± 14*	298 ± 15*	201 ± 12*	385 ± 17*	389 ± 12
Control	202 ± 11	408 ± 14	176 ± 7	290 ± 15	234 ± 8	464 ± 16

Data are expressed as ng of neurotransmitter per g of wet tissue. Each value represents mean ± SEM of 8 determinations.

*$p<0.001$: compared to controls (t-test).

TABLE 3

EFFECT OF IPRINDOLE ON THE LEVELS OF DOPAMINE AND NOREPINEPHRINE IN DIFFERENT REGIONS OF THE MOUSE BRAIN

Dose of Iprindole mg/kg/day	Brain Structure: Cerebral Cortex DA	Cerebral Cortex NE	Mesencephalon DA	Mesencephalon NE	Cerebellum DA	Cerebellum NE
0.5	154 ± 5	266 ± 4	124 ± 5	439 ± 20	193 ± 5	153 ± 6
1.0	166 ± 5	280 ± 10	132 ± 8	479 ± 18	189 ± 5	167 ± 5
2.0	167 ± 6	262 ± 8	143 ± 5	496 ± 22	213 ± 5	168 ± 6
Control	158 ± 5	259 ± 5	146 ± 7	479 ± 15	201 ± 9	158 ± 6

Data are expressed as ng of neurotransmitter per g of wet tissue. Each value represents mean ± SEM of 8 determinations.

t-test analysis revealed no significant differences between treated and control groups.

neither DA nor NE concentrations were significantly modified by iprindole treatment.

DISCUSSION

On the basis of substrate and inhibitor specificities, two types of mitochondrial bound MAO were recognized, types A and B, representing two classes of enzymes with similar characteristics [10,22]. In the brain the predominant MAO form is of the A type [6,22], but since both classes of enzymes occur in the brain, the MAO activity determined is a measure of total enzymatic activity.

The differential distribution of MAO activity agrees with several earlier reports [3, 9, 14, 25] and further supports the suggestion that neurotransmitters are unevenly degraded throughout the brain. However, it is not known if this differential metabolic disposition of biogenic amines is related to differences in the functional activity of the various brain areas of brain analyzed.

Our results clearly demonstrate that MAO activity is markedly inhibited after 120 days of iprindole treatment (Table 1). This inhibition was observed in all regions examined, but showed different characteristics in the different regions. The observations, (1) that the highest degree of MAO inhibition is reached at the lowest dose of iprindole, and (2) that a substantial amount of enzyme activity remains unaffected (an average of 60%), suggest that a specific fraction of the total enzyme activity was inhibited by the iprindole treatment.

In the present study, the effective iprindole concentrations was progressively reduced by mass action due to several factors: dilution of the brain tissue caused by the homogenizing buffer, the media used during isolation of the mitochondrial fraction, and the buffer used in the assay. Since the substrate concentration in the routine standard assay was always higher than that of iprindole, none of the reversible enzyme inhibitors could be detected [17]. These conclusions are further reinforced by the observation that the highest percentage of total MAO inhibition was obtained at the lowest dose of iprindole used. It may therefore be assumed that iprindole inhibits MAO activity by acting as an irreversible inhibitor. However, other mechanisms of action cannot be excluded including, e.g., inhibition of protein synthesis or establishment of a new homeostasis due to the long-term drug administration.

As a consequence of the MAO inhibition, the brain content of 5-HT increased whereas that of 5-HIAA decreased. Since neither DA nor NE levels were modified, a substrate-specific inhibition of MAO may be postulated as has been suggested for other drugs [6, 9, 29]. Further experiments should be undertaken using substrates other than tryptamine, to test this hypothesis.

Assuming a constant efflux rate of 5-HIAA from brain to blood, MAO inhibition should elicit both an increase in 5-HT and a decrease in 5-HIAA levels. Such an inverse relationship between MAO substrate and product was not observed. If iprindole does not affect cerebral disposal of 5-HIAA, other effects besides MAO inhibition must be assumed to influence the 5-HT metabolism, for instance, increased 5-HT synthesis. Further experiments are needed to support this suggestion.

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