TABLE 2. EFFECTS OF SALICYLATE CONGENERS ON THE RESPIRATION OF HUMAN RED CELLS

	Conc (mM)	Glucose utilized dis/min × 10^{-3}	$^{14}CO_2$ Produced dis/min × 10^{-3}	% [1-^{14}C] Glucose converted to $^{14}CO_2$
Control		406 ± 8	22·0 ± 0·3	5·4 ± 0·2
2-Hydroxybenzoate	5	404 ± 3	20·7 ± 0·5	5·1 ± 0·1
	20	349 ± 3	12·0 ± 0·3	3·4 ± 0·1
3-Hydroxybenzoate	5	384 ± 6	21·0 ± 0·4	5·5 ± 0·1
	20	376 ± 11	16·1 ± 0·6	4·3 ± 0·3
4-Hydroxybenzoate	5	380 ± 14	17·3 ± 0·6	4·6 ± 0·3
	20	312 ± 9	13·7 ± 0·8	4·4 ± 0·4
2,4-Dihydroxybenzoate	5	344 ± 7	20·5 ± 1·0	6·0 ± 0·5
	20	216 ± 12	16·3 ± 0·7	7·5 ± 0·5
2,5-Dihydroxybenzoate	5	328 ± 10	28·8 ± 1·7	8·8 ± 0·8
	20	208 ± 15	35·6 ± 2·5	17·1 ± 1·3
2,6-Dihydroxybenzoate	5	334 ± 7	14·5 ± 0·7	4·4 ± 0·1
	20	220 ± 4	6·3 ± 0·2	2·9 ± 0·1
3,4-Dihydroxybenzoate	5	328 ± 10	18·2 ± 0·6	5·5 ± 0·3
	20	280 ± 14	19·4 ± 0·5	6·9 ± 0·6

* Each observation represents the mean of eight estimations ± S.D. Radioactivity is expressed as dis/min × 10^{-3}.

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Effects of monoamine oxidase inhibitors on 5-hydroxytryptamine content in different anatomical areas of dog brain

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ADMINISTRATION of monoamine oxidase inhibitors has been shown to result in the increase of the levels of certain brain amines including 5-hydroxytryptamine, adrenaline, and noradrenaline.[1–5] So far such studies have mostly been conducted on the determination of 5-hydroxytryptamine and

noradrenaline contents of total brain homogenate and subcellular fractions obtained through differential centrifugation of such homogenates. In the present study effects of monoamine oxidase inhibitors were investigated on 5-hydroxytryptamine content and monoamine oxidase activity in different anatomical areas of dog brain in an attempt to show relationship between increase in 5-hydroxytryptamine content with monoamine oxidase inhibition. Studies were conducted with isolated hypothalamus, midbrain, thalamus, medulla, cortex, and cerebellum.

MATERIALS AND METHODS

Healthy dogs weighing between 10 and 15 kg were used for the present study. Six animals were taken for the control group and four for studying the effects of each monoamine oxidase inhibitor. Animals anesthetized either by chloroform or intravenous pentobarbitone (30 mg/kg) were exsanguinated[6] through the carotid artery in order to prevent contamination of brain 5-hydroxytryptamine with that of the blood. The whole brain was taken out and washed repeatedly with cold 0·9% (w/v) saline. Half the brain was used for estimation of 5-hydroxytryptamine and the other half for monoamine oxidase activity.

The hypothalamus, midbrain, thalamus, medulla, cortex, and cerebellum were dissected out immediately and weighed separately. All these brain areas were homogenized in cold acetone (A.R.) in a Potter–Elvehjem homogenizer. The acetone extract was filtered, and the residue was further extracted with an equal volume of cold acetone and filtered again. The combined filtrate was evaporated to dryness at 35° under reduced pressure. The dried residue thus obtained was taken up in normal saline (2 ml/g of wet tissue). The lipids present in the extract were removed by shaking with light petroleum ether (b.p. 40–60°). The clear extracts thus obtained were used for the estimation of 5-hydroxytryptamine by the modified[7] rat uterus bioassay method of Gaddum and Hameed.[8] Atropine at a final concentration of 1×10^{-6} g was present in the bath maintained between 25 and 28°. Abolition of the responses of the rat uterus by the use of 0·2 μg 2-bromolysergic acid (BOL)/ml was indicative of the specificity of 5-hydroxytryptamine responses. The content of 5-hydroxytryptamine was determined by the log–dose response curve, and wherever necessary the priming dose method of Woolley and Campbell[9] was employed.

The values of 5-hydroxytryptamine content obtained by direct bioassay of the tissues extract were found to be identical with those obtained after separation of 5-hydroxytryptamine from these extracts by paper chromatography.[10] Recoveries of 5-hydroxytryptamine added to the brain extracts were quantitative. Monoamine oxidase activity was determined by the conventional Warburg manometric method[11] with brain homogenate in 0·25 M cold sucrose. The enzyme activity was expressed as microliters of oxygen uptake during oxidative deamination of tyramine. Monoamine oxidase inhibitors used in the study are pargyline (Eutonyl, MO-911); pheniprazine (Catron, JB-516); tranylcypromine (Parnate, SKF-385); isocarboxyzid (Marplan); and D-amphetamine.

RESULTS AND DISCUSSION

The values for 5-hydroxytryptamine content determined by rat uterus bioassay method are shown in Fig. 1. Hypothalamus contained the highest concentration of 5-hydroxytryptamine. Midbrain, thalamus, and medulla also contained appreciable amounts, while low amounts were present in cortex. The cerebellum was more or less devoid of 5-hydroxytryptamine. Our results are in agreement with those reported by other investigators,[12, 13] who used chemical methods for the determination of 5-hydroxytryptamine. Monoamine oxidase activity on the other hand was found to be nearly uniform in all the areas of the brain (Fig. 2). Cerebellum, although devoid of 5-hydroxytryptamine content, possessed significant enzyme activity which was almost equal to that in hypothalamus, having a maximal concentration of 5-hydroxytryptamine (Fig. 1). Similar results have been reported by Bogdansky *et al.*,[14] who used 5-hydroxytryptamine utilization as an index of the enzyme activity. These investigators, however, found that hypothalamus contained three times as much activity as the other areas. Substrate specificity may account for such a difference. In the present study, use of different anesthetic agents was found to have no influence on 5-hydroxytryptamine content and monoamine oxidase activity in the different areas of brain, since identical values were obtained by using either chloroform or intravenous phenobarbitone.

Intravenous administration of pargyline at a dose of 2 mg/kg (animal sacrificed after 24 hr) increased 5-hydroxytryptamine content in all the areas of the brain (Fig. 1). Approximately threefold increase was observed in the hypothalamus. Such an increase in the content of 5-hydroxytryptamine was of the order of 2·31, 1·49, 2·19, and 2·33 times the normal in midbrain, thalamus, medulla, and

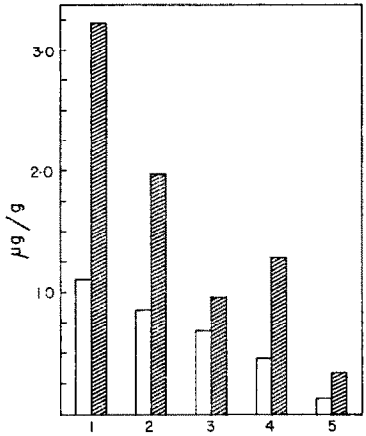

FIG. 1. Effect of intravenous administration of pargyline (2 mg/kg) on 5-hydroxytryptamine content of hypothalamus (1), midbrain (2), thalamus (3), medulla (4), and cortex (5). The bioassay procedure is as given in the text; unshaded bars represent control values from untreated animals; hatched bars represent values from pargyline-treated animals.

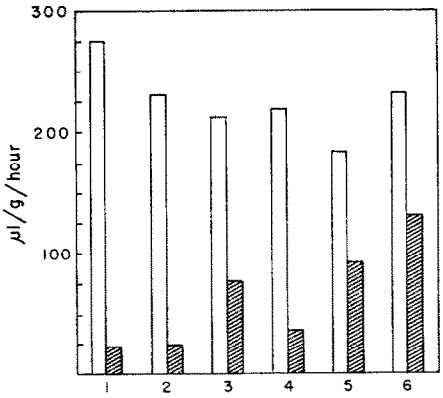

FIG. 2. Effect of intravenous administration of pargyline (2 mg/kg) on monoamine oxidase activity of hypothalamus (1), midbrain (2), thalamus (3), medulla (4), cortex (5), and cerebellum (6). The assay procedure is as given in the text; unshaded bars represent control values from untreated animals; hatched bars represent values from pargyline-treated animals.

cortex respectively. Similarly, intravenous administration of 3 mg pheniprazine/kg (animal killed after 1·5 hr) and 5 mg tranylcypromine/kg (animal killed after 16 hr) caused a highly significant rise in the content of 5-hydroxytryptamine in cortex, medulla, hypothalamus, and midbrain (Table 1). Such an increase in thalamus was less significant. Unlike pheniprazine and tranylcypromine, administration of 50 mg isocarboxyzid/kg (animal sacrificed after 6 hr) caused significant rise of 5-hydroxy-

tryptamine content only in medulla and hypothalamus. Isocarboxyzid administration caused slight increase in midbrain and thalamus, whereas no increase could be observed in the cortex.

Effect of intravenous administration of pargyline (2 mg/kg) on monoamine oxidase activity of different areas of the brain is represented in Fig. 2. The percentage of enzyme inhibition was found to be 92, 90, 64, 85, 50, and 43 in hypothalamus, midbrain, thalamus, medulla, cortex, and cerebellum

TABLE 1. EFFECT OF MONOAMINE OXIDASE INHIBITORS ON 5-HYDROXYTRYPTAMINE CONTENT

Brain regions	5-Hydroxytryptamine (μg/g)				
	Control	Pargyline (25 mg/kg)	Pheniprazine (3 mg/kg)	Tranylcypro-mine (5 mg/kg)	Isocarboxyzid (50 mg/kg)
Hypothalamus	1·09 ± 0·08*	3·22 ± 0·05 P <0·001	1·55 ± 0·05 P <0·001	1·54 ± 0·05 P <0·01	1·57 ± 0·09 P <0·01
Midbrain	0·86 ± 0·04	1·96 ± 0·07 P <0·001	1·23 ± 0·04 P <0·001	1·21 ± 0·07 P <0·001	0·99 ± 0·05 P <0·05
Thalamus	0·64 ± 0·06	0·95 ± 0·03 P <0·001	0·90 ± 0·05 P <0·01	0·71 ± 0·03 P <0·4	0·71 ± 0·04 P <0·4
Medulla	0·46 ± 0·03	1·27 ± 0·09 P <0·001	0·70 ± 0·03 P <0·001	0·69 ± 0·04 P <0·01	0·71 ± 0·04 P <0·001
Cortex	0·13 ± 0·02	0·31 ± 0·01 P <0·001	0·21 ± 0·01 P <0·01	0·24 ± 0·02 P <0·01	0·12 ± 0·02 P <0·08

Note: significance was determined between untreated and treated animals by t-test.
* Mean ± standard error.

respectively. As is evident from Table 2, inhibition of monoamine oxidase in all the areas of the brain was also observed after administration of pheniprazine (3mg/kg), tranylcypromine (5mg/kg) and isocarboxyzid (50 mg/kg). Significant decrease in the enzyme activity was observed in medulla, hypothalamus, midbrain, and thalamus as compared to that observed in cortex and cerebellum. The decrease of monoamine oxidase inhibition ranged from 43–78, 48–77, and 82–91 per cent with pheniprazine, tranylcypromine, and isocarboxyzid respectively (Table 2).

TABLE 2. EFFECT OF MONOAMINE OXIDASE INHIBITORS ON ENZYME ACTIVITY

Brain regions	Monoamine oxidase activity μl/g/hr				
	Control	Pargyline (25 mg/kg)	Pheniprazine (3 mg/kg)	Tranylcypro-mine (5 mg/kg)	Isocarboxyzid (50 mg/kg)
Hypothalamus	274 ± 6·69*	23 ± 2·3 P <0·001	76 ± 2·6 P <0·001	80 ± 2·1 P <0·001	26 ± 0·34 P <0·001
Midbrain	229 ± 7·17	22 ± 1·1 P <0·001	63 ± 1·4 P <0·001	81 ± 6·9 P <0·001	23 ± 1·4 P <0·001
Thalamus	211 ± 5·46	76 ± 3·1 P <0·001	70 ± 1·3 P <0·001	70 ± 2·1 P <0·001	23 ± 2·8 P <0·001
Medulla	217 ± 6·70	33 ± 1·2 P <0·001	48 ± 1·99 P <0·001	47 ± 2·9 P <0·001	22 ± 2·8 P <0·001
Cortex	182 ± 6·20	90 ± 4·27 P <0·001	104 ± 5·2 P <0·001	100 ± 2·5 P <0·001	33 ± 2·5 P <0·001
Cerebellum	230 ± 9·21	129 ± 0·71 P <0·001	102 ± 3·5 P <0·001	104 ± 4·9 P <0·001	38 ± 5·4 P <0·001

Note: significance was determined between untreated and treated animals by t-test.
* Mean ± standard error.

In the present study administration of D-amphetamine (20mg/kg) given subcutaneously in three divided doses at an interval of 1 hr (animals killed 4·5 hr after the first injection) was found to have no effect on the concentration of 5-hydroxytryptamine and monoamine oxidase activity in the different areas of the brain. It seems that the weak monoamine oxidase inhibitory property of D-amphetamine[15] is presumably responsible for such results. However, administration of D-amphetamine caused marked central excitement in these animals.

The results demonstrate unequivocally that administration of monoamine oxidase inhibitors shows regional variations in terms of 5-hydroxytryptamine content and monoamine oxidase inhibition in dog brain. It was interesting to note that monoamine oxidase activity of the cortex, which is least affected by the administration of monoamine oxidase inhibitors, showed highly significant and maximal relative increase in 5-hydroxytryptamine content. This was observed with all the inhibitors except D-amphetamine. Difference in the degree of monoamine oxidase inhibition, in areas having similar enzyme activity, points toward different regional effects of monoamine oxidase inhibitors, owing to the multiplicity of the enzyme monoamine oxidase and the nature of its active site(s). In the present study, no definite correlation could be observed between the increase in 5-hydroxytryptamine content and monoamine oxidase inhibition. It could therefore be assumed that additional pathways responsible for the destruction and synthesis of 5-hydroxytryptamine are involved in these different anatomical areas of the dog brain.

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