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INHIBITION OF AROMATIC L-AMINO ACID DECARBOXYLATION BY DECABORANE*

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Abstract—The effect of decaborane ($B_{10}H_{14}$) upon the 5-hydroxytryptamine (5HT) content and the aromatic L-amino acid decarboxylase activity in rat brain was studied. Also examined was the effect of decaborane upon the 5-hydroxytryptophan (5HTP)-induced increase in rat brain 5HT.

Decaborane diminished the 5HT content of rat brain and also abolished the 5HTP-induced 5HT elevation. This boron hydride inhibited decarboxylase in a rat brain homogenate system prepared from animals injected with decaborane. Decarboxylase was also inhibited in control rat brain homogenates to which decaborane was added *in vitro*.

From the data it was concluded that probably one of the mechanisms of action of decaborane is inhibition of decarboxylase, but a second component may be a release phenomenon.

MAMMALIAN aromatic monoamines such as dopamine, 5-hydroxytryptamine, and tyramine are synthesized *in vivo*, for the most part, by decarboxylation of the corresponding amino acids, dihydroxyphenylalanine, 5-hydroxytryptophan, and tyrosine respectively. Inhibition of the enzyme responsible for this decarboxylation, aromatic L-amino acid decarboxylase, should produce a depletion of endogenous aromatic monoamines *in vivo*. Hydrazine derivative inhibitiors of decarboxylase such as Ro 4-4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl hydrazine] produce some depletion of 5-hydroxytryptamine, dopamine, and norepinephrine.[1] It has now been generally established that α-methylated aromatic amino acids, such as α-methyldopa, deplete amines as a result of conversion to α-methyl amines, which then lower endogenous amines by displacing them from storage sites rather than by inhibition of decarboxylase.[2]

The present study was undertaken to investigate the effect of decaborane ($B_{10}H_{14}$) on the biosynthesis of 5-hydroxytryptamine (5HT) from its precursor, 5-hydroxytryptophan.

METHODS

Male Sprague–Dawley rats (200–300 g) were used in this study.

In the experiment in which DL-5-hydroxytryptophan (5HTP) was injected, the following protocol was used. The animals were injected intraperitoneally with

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N-methyl-4-benzyl propynylamine HCl (MO-911) at a dose of 100 mg/kg. Sixteen hours after MO-911, 15 mg decaborane (dissolved in corn oil) per kg was administered; 4 hr later, 100 mg 5HTP (suspended in 0·1% methylcellulose) was given per kg. Controls were injected with appropriate solvents. One hour after 5HTP administration the animals were sacrificed and the brains removed and quick-frozen in liquid nitrogen; 5HT was assayed by the method of Snyder *et al.*[3] within 3 days after sacrifice.

The time course of brain serotonin depletion was determined by administering 15 mg decaborane/kg to rats and assaying at various time intervals thereafter for 5HT as described above.

Assessment *in vitro* of aromatic L-amino acid decarboxylase was made by the method of Kuntzman *et al.*[4] in rat brain homogenate. Animals were injected with decaborane as above and sacrificed at intervals. Decarboxylase activity was then assayed. Additionally, various concentrations of decaborane (in 1 M phosphate buffer, pH 8) were added to control brain homogenates and decarboxylase assayed as above.

RESULTS

Within 15 min after injection of the MO-911-pretreated animals with 5HTP, they became ataxic and there was profuse salivation. Those that received MO-911, 5HTP, and decaborane were only slightly sedated but could be easily aroused and otherwise appeared to be normal.

Table 1 shows 5HT content of whole rat brain after treatment. Decaborane prevented the three-fold rise in 5HT produced by the combination of monoamine oxidase

TABLE 1. LEVELS OF 5HT IN WHOLE RAT BRAIN WITH AND WITHOUT TREATMENT

	(μg/g ± S.D.)	(% of control)
Control	0·41 ± 0·03	
MO-911 + decaborane + 5HTP	0·44 ± 0·09	107
MO-911 + 5HTP	1·37 ± 0·49	334
MO-911 + decaborane	0·49 ± 0·04	120
Decaborane + 5HTP	0·20 ± 0·04	49
MO-911	0·69 ± 0·10	168
Decaborane	0·16 ± 0·02	39
5HTP	0·64 ± 0·16	156

Each group contained ten animals. The animals were pretreated with MO-911 (100 mg/kg in saline). Sixteen hours after MO-911 was administered, decaborane was injected i.p. (15 mg/kg in corn oil). Four hours after decaborane, 5-hydroxytryptophan (100 mg/kg in 0·1% methylcellulose) was given. One hour after 5-HTP administration, the animals were sacrificed and whole-brain 5HT assayed. Control groups were given appropriate solvents.

inhibition (by MO-911) and subsequent administration of exogenous 5HTP. MO-911 prevented the fall in brain 5HT mediated by decaborane.

Treatment with decaborane alone lowered the 5HT content to 39 per cent of control values. When 5HTP was given to decaborane-treated rats, whole-brain 5HT was lowered to 49 per cent of control levels in spite of the plethora of 5HTP available for 5HT synthesis. Finally, as expected, 5HTP alone produced a rise of 5HT to 156 per cent of controls.

After 15 mg decaborane/kg, 5HT levels declined fairly rapidly, reaching greatest depletion within 24 hr. The brain concentration of 5HT then began to replete slowly

so that within 168 hr after treatment the levels were 75 per cent of control value, as shown in Fig. 1.

Also shown in Fig. 1 is the time course of aromatic L-amino acid decarboxylase inhibition after decaborane treatment. This enzyme was rapidly, and virtually com-

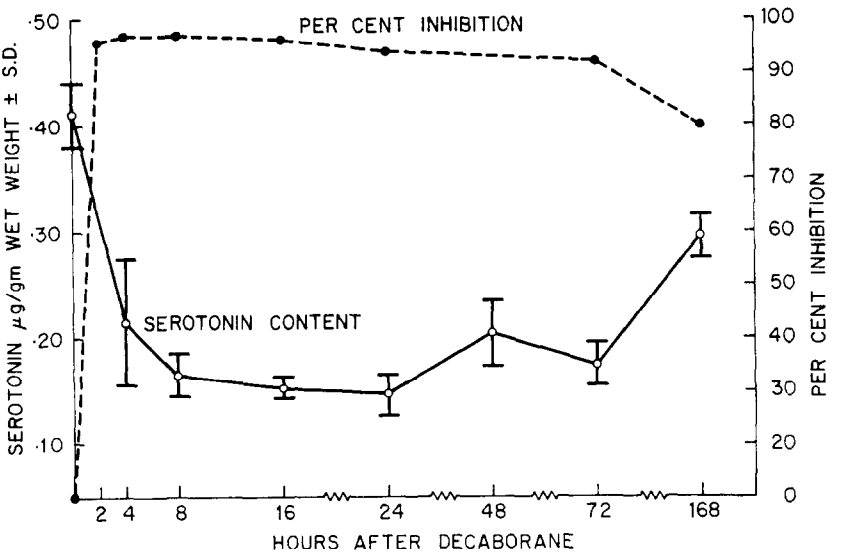

FIG. 1. 5HT content of whole rat brain is shown on the left. Represented on the right is per cent inhibition of formation of 5HT from 5HTP *in vitro* by whole rat brain homogenate. Decaborane was administered i.p. (15 mg/kg).

pletely, inhibited within 2 hr after treatment. At 24 hr after decaborane, enzyme activity started returning; but in 168 hr post-treatment, considerable inhibition remained.

When decaborane was added to the enzyme assay system, decarboxylase activity was almost completely blocked at a concentration of 10^{-6} M, as shown in Table 2. Even at a concentration of 10^{-8} M there was still some inhibition.

TABLE 2. AMOUNT OF 5HT SYNTHESIZED FROM 5HTP BY RAT BRAIN HOMOGENATE WITH AND WITHOUT DECABORANE

Decaborane concentration (M)	(μg 5HT synthesized/ g brain tissue/hr)
0	74·9
10^{-4}	2·33
10^{-6}	2·19
10^{-8}	39·1

Decaborane, in the concentration shown, was added to the incubation medium which consisted of 18·2 μmoles 5HTP, 0·3 μmoles pyridoxal phosphate, 1·2 m-moles phosphate buffer, and 0·5 m-moles sucrose. Final volume was 14 ml. Each group consisted of nine experiments.

DISCUSSION

The results tend to confirm the previous observation that decaborane may inhibit L-amino acid decarboxylase.[5] The 5HT levels observed after the combined treatment with 5HTP, MO-911, and decaborane were the same as when MO-911 and decaborane were given in combination. This would seem to indicate that no 5HT was being formed from the exogenous 5HTP and that the decarboxylase was inhibited. The inhibition by decaborane of the large increase produced by the combination of MO-911 and 5HTP was of somewhat greater magnitude than that shown for several decarboxylase inhibitors by Hansson *et al.*[6] In their investigation the most active compound inhibiting the formation of 5HT was NSD 1034 (3-hydroxybenzylamine trihydrogen phosphate). With this compound there was still an increase in 5HT of 185 per cent after 5HTP loading, when approximately the same conditions as in the present experiment were used.

The 5HTP-induced increase of 5HT in the brain of mice was greatly inhibited by large doses of Ro 4-4602.[1] Ro 4-4602 decreased endogenous 5HT, which effect can be explained by decarboxylase inhibition. Decaborane also completely inhibited the increase of 5HT mediated by exogenous 5HTP. After treatment with decaborane and 5HTP, the levels of 5HT were lowered to the same extent as that seen when decaborane is given alone, whereas when 5HTP was given alone, 5HT levels were 156 per cent of control values, as has been previously observed.[7]

The decrease in 5HT levels after decaborane and the inhibition of the rise in 5HT levels after 5HTP loading could be considered indications of inhibition of decarboxylase by this compound. Alternatively, a mechanism of blockade of storage by decaborane would have to be considered.

Other quite strong decarboxylase inhibitors, such as some of the "NSD" compounds, do not lower amine levels.[8, 9] Pletscher *et al.* found that Ro 4-4602, a very specific inhibitor of decarboxylase, decreased endogenous rat brain 5HT about 50 per cent at maximal depletion.[1, 10] In the present experiment, 5HT was reduced to 37 per cent of control levels at maximal depletion; i.e. 24 hr after treatment with decaborane.

A comparison of the time course of 5HT depletion and decarboxylase inhibition produced by decaborane indicates that when decarboxylase activity began to return, 5HT levels began to rise. Both 5HT depletion and decarboxylase inhibition were slow to be reversed; neither normal 5HT level nor enzyme activity has been restored after 168 hr. This contrasts with the time course of restoration of 5HT and normal enzyme activity after treatment with α-methyldopa or Ro 4-4602. In both cases, decarboxylase activity began to return within 2 hr after injection, and control 5HT levels were reached within about 24 hr post-treatment.[10]

That the lowered levels of 5HT after decaborane are not solely related to the inhibition of 5HTP decarboxylase may also be inferred from the data, which suggest that 5HT levels after administration of 5HTP, MO-911, and decaborane, or after decaborane and MO-911, may be due to the interference with decaborane-induced depletion of 5HT by MO-911. In the absence of MO-911, decaborane significantly decreased the levels of 5HT to the same degree either in the presence or absence of 5HTP. Previously it was shown that MO-911 prevented the depletion of norepinephrine by decaborane.[11] This finding might be explained on the basis of interference by MO-911 of amine release, since decaborane, in addition to decarboxylase inhibition,

also increases the rate of release of at least one amine, norepinephrine, from isolated nerve vesicles.[12]

Uptake seems to be unaffected by decaborane, however. Euler and Lishajko showed that the decaborane-depleted norepinephrine stores in rabbit heart are refilled to near normal capacity by injection of norepinephrine.[13]

The combination of the release of norepinephrine from isolated nerve vesicles and the inhibition of decarboxylase by decaborane is similar to the manner in which α-methyldopa is believed to deplete endogenous biogenic amines; that is, not only by inhibition of decarboxylase but also by a release mechanism. Unlike Ro 4-4602, decaborane produces sedation, which action is also similar to α-methyldopa.

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