Biochemical Pharmacology, 1966, Vol. 15, pp. 481–487. Pergamon Press Ltd., Printed in Great Britain.

CONVERSION OF INDOLYLALKYLHYDROXYLAMINES TO STIMULANT AMINES *IN VIVO*

A. W. LESSIN, R. F. LONG and M. W. PARKES

Pharmacological Laboratory, Research Department,
Roche Products Ltd., Welwyn Garden City

(*Received* 24 *August* 1965; *accepted* 7 *October* 1965)

Abstract—The stimulant effects due to several indolylalkylhydroxylamines in mice were shown to resemble closely in degree and in time course those of the corresponding indolylalkylamines. The hydroxylamine derivatives were shown to be inactive as inhibitors of monoamine oxidase, and of the uptake of amines *in vitro*, properties which had been suggested as responsible for the stimulant actions of the amines. Since the tissues of animals treated with the hydroxylamine derivatives were shown to contain the corresponding amine and tissue homogenates incubated with the hydroxylamines rapidly reduced them, it was concluded that the stimulant properties observed were due to the formation of indolylalkylamines *in vivo*.

THE CENTRAL stimulant properties of α-alkylindolylalkylamines in mice have been ascribed to the potentiation of endogenous stimulant amines both by interfering with their storage and inhibiting their destruction by monoamine oxidase.[1–3] A number of corresponding indolylalkylhydroxylamine derivatives has now been prepared[4] and their properties are reported here.

METHODS

Stimulant activities, including tremor, hyperthermia and mydriasis, were detected and measured in mice by the methods described in earlier papers.[1, 5–7] Stimulant effects of the compounds in mice additionally treated with other drugs were measured as tremor.[6] Additional treatments were: (1) pretreatment with pargyline hydrochloride (three doses of 75 mg/kg intraperitoneally at 1½ hr intervals, the last being 1½ hr before administration of the test compound): or (2) treatment with reserpine, 2 mg/kg intraperitoneally, 15 min later: or (3) treatment with 5-hydroxytryptophan, (HTP) 50 mg/kg intraperitoneally 20 min later.

Inhibition of monoamine oxidase

The enzyme preparation consisted of the mitochondrial fraction of a guinea pig liver homogenate, washed three times with water and resuspended in M/15 phosphate buffer, pH 7·2. The substrate used was 5-hydroxytryptamine (HT), 2×10^{-3} M, and enzyme activity was measured manometrically.

Inhibition of the uptake of HT

The method is described in a previous paper.[2]

Estimation of α-alkyltryptamines in tissues

Tissue homogenates in water were made alkaline (pH ~ 12·0) with aqueous sodium hydroxide and extracted with ether. Basic materials were back extracted into 0·1 N HCl and estimated either by the xanthydrol reaction[8] or by the fluorimetric method for tryptamine.[9]

Qualitative investigation of urinary indoles

The compounds under investigation were given to groups of four mice at 20 mg/kg intraperitoneally. Urine was collected for 24 hr and aliquots were examined by paper chromatography in butanol/acetic acid/water, 8:1:1, or butanol saturated with 2N HCl. Indoles were revealed by dipping in dimethylaminocinnamaldehyde reagent.[10]

Qualitative investigation of tissue indoles

The pH of tissue homogenates from studies *in vivo* or *in vitro* was adjusted to 9·0 and they were extracted with benzene. Benzene extracts were concentrated and suitable aliquots examined by chromatography in the above systems.

The amines used in this study were prepared by Drs. B. Heath-Brown and P. G. Philpott of Roche Products Ltd.[11]

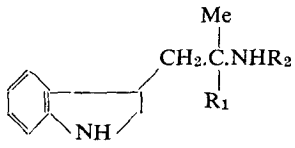


FIG. 1.

Ro 3-0926, $R_1 = R_2 = H$ (α-methyltryptamine)
Ro 3-2040, $R_1 = H$; $R_2 = OH$
Ro 3-1638, $R_1 = Me$; $R_2 = H$ (α,α-dimethyltryptamine)
Ro 3-2099, $R_1 = Me$; $R_2 = OH$

RESULTS

Stimulant properties

The indolylalkylhydroxylamines showed stimulant properties in mice very closely resembling those of the corresponding indolylalkylamines. Table 1 gives the relative activities of two hydroxylamine derivatives and the corresponding amines (Fig. 1) determined by comparative assays, for stimulant properties in potentiating tremors due to HTP (inhibition of monoamine oxidase *in vivo*) and in producing tremors when followed by reserpine.

Table 2 indicates the similarities in stimulant properties between several indolylalkylhydroxylamines and the corresponding amines.

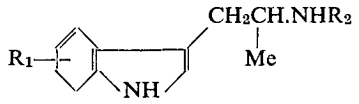
Evidence for conversion

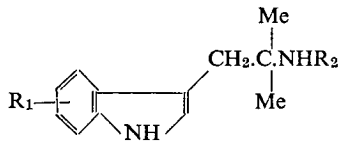
In vivo. Figure 2 shows the time course of tremors due to α-methyltryptamine and the corresponding hydroxylamine (Fig. 1) in mice. Their similarity suggested that the activity of the hydroxylamine could be due to conversion to the amine *in vivo*. To examine this possibility this hydroxylamine, Ro 3–2040, 3-(2-hydroxylaminopropyl)

TABLE 1. STIMULANT PROPERTIES OF INDOLYLALKYLHYDROXYLAMINES AND CORRESPONDING AMINES IN MICE

Effect	Relative activity (i.p. route)			
	Ro 3–0926	Ro 3–2040	Ro 3–1638	Ro 3–2099
Stimulation without pretreatment				
Tremors	1·0	0·84 (0·65–1·15)	~0·1	
			1·0	1·42 (0·48–4·2)
Hyperthermia (at 30°)	1·0	1·08 (0·86–1·37)	0·116 (0·007–0·13)	
			1·0	1·13 (0·9–1·42)
Mydriasis	1·0	1·13 (0·65–1·9)		
Production of tremors after pretreatment with pargyline (3 doses of 75 mg/kg i.p. at 1½ hr intervals, the last being 1½ hr before assay)	1·0	~1	~0·5	
			1·0	0·86 (0·37–2·0)
Tremors with HTP 50 mg/kg i.p. 20 min after pretreatment with compound	1·0	0·98 (0·7–1·35)	~0·33	
			1·0	0·89 (0·63–1·26)
Tremors with reserpine 2 mg/kg	1·0	1·18 (0·96–1·45)	0·25	
i.p. 15 min pretreatment with compound			1·0	0·96 (0·81–1·13)
Acute LD_{50} mg/kg	162 (156–170)	~175	147 (136–159)	100 (87–115)

TABLE 2. STIMULANT PROPERTIES OF INDOLYLALKYLHYDROXYLAMINES AND CORRESPONDING AMINES IN MICE





	R_2 = H		R_2 = OH		R_2 = H		R_2 = OH	
R_1	Ro 3–	Class	Ro 3–	Class	Ro 3–	Class	Ro 3–	Class
H	0926	A+++	2040	A+++	1638	B+++	2099	B+++
6-Me					2048	B+	2148	B+
5-MeO					2176/1	0	2175	0
6-MeO	1890	A++	2119	A+				
5-Cl	1985	A++	2189	A++	2192/1	0	2182	0

Class A. Tremors in doses up to 100 mg/kg intraperitoneally.
B. Tremors in doses up to 20 mg/kg intraperitoneally in mice pretreated with repeated doses monoamine oxidase inhibitor.
0. Inactive.
+, ++, +++ } Degree of activity within class.

indole, was given to mice intraperitoneally at 20 mg/kg. Kidney and brain homogenates were made using the pooled organs from 10 mice killed 30 min after administration of the compound. In extracts of these homogenates the hydroxylamine was absent and the only indole which could be detected by paper chromatography was

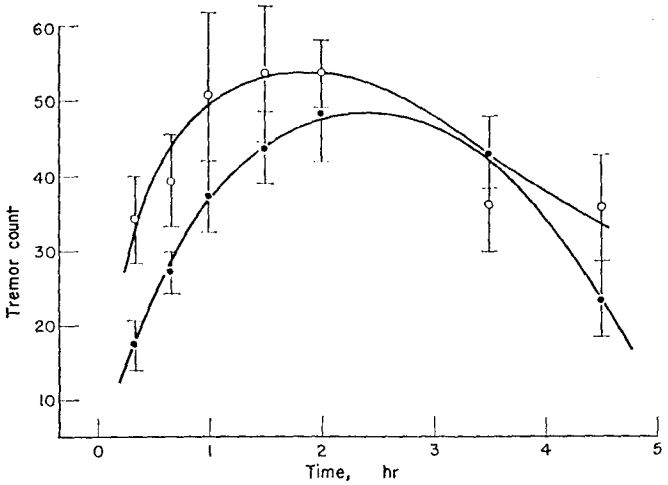


FIG. 2. Tremor count in groups of 10 mice at various intervals after intraperitoneal injection, at time 0,

●: α-Methyltryptamine, 10 mg/kg and ○: 3-(2-Hydroxylaminopropyl)indole, 10 mg/kg.

TABLE 3. INDOLYLALKYLAMINE LEVELS IN THE BRAINS OF MICE INJECTED INTRAPERITONEALLY WITH INDOLYLALKYLHYDROXYLAMINES OR THE CORRESPONDING AMINES

Time after injection (hr)	Amine concentrations (μg/g brain) after injecting 20 mg/kg of			
	Ro 3–2040	Ro 3–0926	Ro 3–2099	Ro 3–1638
1	7·0	5·1	7·4	4·2
2	5·4	5·2	6·3	5·7
4	2·3	3·3	2·9	4·3

Each value is the mean of 2 separate determinations on 4 pooled brains. Indolylalkylamines were measured colorimetrically by the xanthydrol reaction.

α-methyltryptamine. Similar experiments using Ro 3–2099, 3-(2-hydroxylamino-2'-methyl propyl) indole, showed that tissues 30 min after administering the compound contained only the corresponding amine α,α-dimethyltryptamine (Fig. 1).

Tables 3 and 4 compare the levels of indolylalkylamines found in the brains of mice and rats given these indolylalkylhydroxylamines with the levels found after giving the amines themselves.

It will be seen that the appearance and disappearance of the amines were similar, whether amine or corresponding hydroxylamine was given and that these also correspond to the time course of the stimulant effects observed, as already shown for α-methyltryptamine.[1]

TABLE 4. LEVELS OF α-METHYLTRYPTAMINE IN THE BRAINS OF RATS INJECTED INTRAPERITONEALLY WITH α-METHYLTRYPTAMINE OR THE CORRESPONDING HYDROXYLAMINE

Time after injection (hr)	Amine concentration (μg/g. brain) after injecting 20 mg/kg. of Ro 3–2040	Ro 3–0926	
½	3·5 ± 0·3	2·8 ± 0·24	P > 0·05
1	4·9 ± 0·27	3·9 ± 0·31	P = 0·05
2	2·3 ± 0·11	2·8 ± 0·23	P > 0·05
4	1·1 ± 0·16	1·8 ± 0·5	
6	0·6 ± 0·20	1·5 ± 0·19	

Each value is the mean of 4 determinations each being made in duplicate on a single rat brain. Standard errors are given. The α-methyltryptamine was measured fluorimetrically.

During the first hour after administration of the hydroxylamines stimulation was somewhat greater than after equivalent doses of the amines (Fig. 2). Similar differences were observed in brain amine levels (Tables 3 and 4).

Mice were given indolylalkylhydroxylamines or the corresponding amines and the urinary indoles compared by paper chromatography. In each case the metabolites from the hydroxylamine were identical with those from the corresponding amine (Fig. 3).

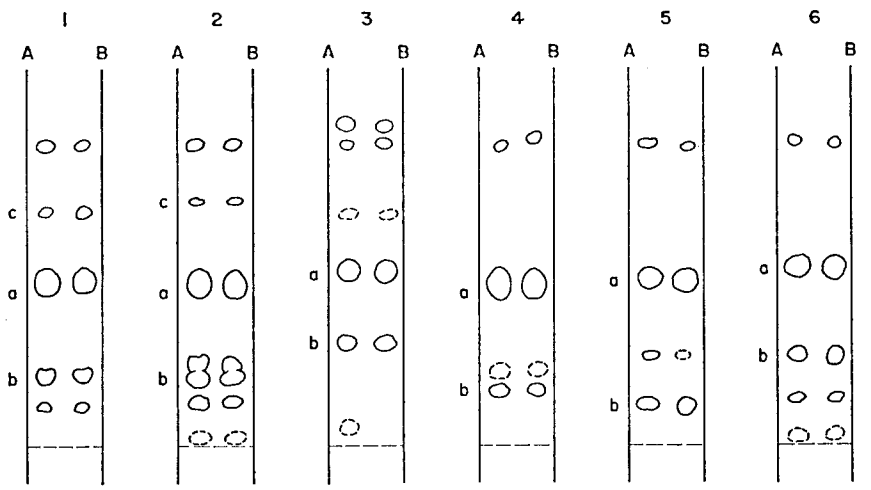


FIG. 3. Diagrams of pairs of paper chromatograms, run in butanol/hydrochloric acid on Whatman No. 1 paper, each comprising samples of pooled urine from 4 mice treated, A, with an indolylalkylamine or B, with the corresponding indolylalkylhydroxylamine.

1. Ro 3-0926 and Ro 3-2040
2. Ro 3-1638 and Ro 3-2099
3. Ro 3-1985 and Ro 3-2189
4. Ro 3-2048 and Ro 3-2148
5. Ro 3-2192/1 and Ro 3-2182
6. Ro 3-2176/1 and Ro 3-2175

Identified spots are: a. urea; b. indolylalkylamine and c. glucuronide of 6-hydroxylated indolylalkylamine.[10]

In vitro. Homogenates of various mouse tissues (200 mg/ml in M/15 phosphate buffer, pH 7·4) were incubated in air for 30 min at 37° with the hydroxylamines Ro 3–2040 and Ro 3–2099 (10 μg/ml). Extracts made as described above showed that liver, brain, kidney, spleen and heart all converted the hydroxylamine completely to the amine. No conversion occurred in homogenates of intestine or in plasma or whole blood.

In vitro activities. None of the indolylalkylhydroxylamines showed any inhibitory properties towards monoamine oxidase *in vitro* in concentrations up to 2×10^{-3} M., although the corresponding indolylalkylamines were appreciably active (Table 5). The hydroxylamine derivatives were weakly effective as inhibitors of the uptake of HT by blood platelets, concentrations of 5×10^{-4} M. being required for 50 per cent inhibition with Ro 3–2040 and Ro 3–2099; they were thus not more than one-tenth as active as the corresponding tryptamine derivatives.[2]

TABLE 5. INHIBITION OF MONOAMINE OXIDASE BY INDOLYLALKYLAMINES *in vitro*

Compound	Molar concn. for 50% inhibition of enzyme
Ro 3–0926	3×10^{-5}
Ro 3–1890	8×10^{-5}
Ro 3–1985	2×10^{-4}
Ro 3–1638	$1{\cdot}5 \times 10^{-4}$
Ro 3–2048	2×10^{-4}

Enzyme: guinea pig liver mitochondrial fraction. Substrate: serotonin creatinine sulphate 2×10^{-3} M. For structures of compounds see Table 2.

DISCUSSION

The results presented show that the stimulant properties in mice of a series of indolylalkylhydroxylamines are closely similar to those of the corresponding indolylalkylamines, both in degree of activity and in time course. *In vitro* studies show that the hydroxylamine derivatives lack the inhibitory properties of the amines towards monoamine oxidase and the uptake of HT by blood platelets, which were suggested as the basis for the mechanism of central stimulant action by the tryptamine derivatives.[1] Injection of a hydroxylamine derivative was followed by appearance in the brain of the corresponding amine to an extent, and with a time course, adequate to account for the stimulant effects observed.

We may thus conclude that the stimulant effects seen with the hydroxylamine derivatives are due to their conversion, in the body, to the corresponding amines. This has been shown to occur *in vitro* in a number of tissues. Whether this is an enzyme-catalysed reaction or the result of chemical interaction with a tissue component has not been investigated.

It has recently been reported that a number of arylalkylhydroxylamines, including those corresponding to amphetamine and α-methyltryptamine, possess stimulant properties resembling those of the amines.[13] A similar conversion to the amine *in vivo*, to that evidenced here, could also account for these observations.

REFERENCES

1. A. W. Lessin, R. F. Long and M. W. Parkes, *Brit. J. Pharmac.* **24**, 49 (1965)
2. A. W. Lessin, R. F. Long and M. W. Parkes, *Brit. J. Pharmac.* **24**, 68 (1965)
3. A. W. Lessin, R. F. Long and M. W. Parkes, to be published.
4. A. Cohen and B. Heath-Brown, *J. Chem. Soc.* 7179 (1965).
5. A. W. Lessin and M. W. Parkes, *Brit. J. Pharmac.* **12**, 245 (1957).
6. A. W. Lessin, *Biochem. Pharmac.* **2**, 290 (1959).
7. M. W. Parkes and A. W. Lessin, *Acta Internat. Meeting on Techniques for the Study of Psychotropic Drugs, Bologna* (Ed. G. Tonini). p. 57. Societa Tipografica Modenese (1960).
8. H. Weissbach, W. King, A. Sjoerdsma and S. Udenfriend, *J. biol. Chem.* **234**, 81 (1959).
9. A. Sjoerdsma, J. A. Oakes, P. Zaltzman and S. Udenfriend, *J. Pharmac.* **126**, 217 (1959).
10. J. Harley-Mason and A. A. P. G. Archer, *Biochem. J.* **69**, 60 (1958).
11. B. Heath-Brown and P. G. Philpott, *J. Chem. Soc.* 7165 (1965).
12. S. Szara, *Experientia*, **17**, 76 (1961).
13. F. Benington, R. D. Morin and L. C. Clark, *J. Med. Chem.* **8**, 100 (1965).