Monoamine Oxidase Inhibiting Activity of a Series of (±)-4-Methoxy-β-hydroxyphenethylamines

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Abstract □ The synthesis and selected pharmacological testing of (±)-4-methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-aminoethanol] and its *N*-methylated derivatives are presented. Members of this series were found to exert partial prevention of reserpine-induced hypothermia in mice and to inhibit monoamine oxidase in Warburg studies. Activity was essentially dose dependent. The secondary amine was the most active member of the series. The tertiary amine was least active, and the primary amine exhibited intermediate activity.

Keyphrases □ (±)-4-Methoxy-β-hydroxyphenethylamine and *N*-methyl derivatives—synthesis, screened for monoamine oxidase inhibition □ Monoamine oxidase inhibition—synthesis and screening of (±)-4-methoxy-β-hydroxyphenethylamine and *N*-methyl derivatives □ β-Hydroxyphenethylamines, 4-methoxy and *N*-methyl derivatives—synthesis, screened for monoamine oxidase inhibition

The cactus genus *Coryphantha* is known to produce a variety of β-hydroxyphenethylamines (1–7). 4-Methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-aminoethanol], for instance, has been detected in extracts of *Coryphantha cornifera* (DC.) Br. and R. var. *echinus* (Engelm.) L. Benson (1). This compound and its *N*-methylated derivatives have been synthesized and used in this laboratory as chromatographic references when screening extracts of *Coryphantha* species for the presence of alkaloids.

Prior to its detection in a plant system, 4-methoxy-β-hydroxyphenethylamine was reported to be a weak vasoconstrictor and, in large doses, a cardiac depressant (8). The lack of additional reports concerning the pharmacology of this compound and its *N*-methyl homologs prompted a pharmacological investigation. Preliminary studies of hexobarbital sleeping times in mice suggested the possibility of monoamine oxidase inhibition. The present report describes the monoamine oxidase-inhibiting activity of (±)-4-methoxy-β-hydroxyphenethylamine, (±)-*N*-methyl-4-methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-(methylamino)ethanol], and (±)-*N,N*-dimethyl-4-methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-(dimethylamino)ethanol].

EXPERIMENTAL

Synthesis—Synthesis of racemic 4-methoxy-β-hydroxyphenethylamine hydrochloride (mp 168–169°), *N*-methyl-4-methoxy-β-hydroxyphenethylamine hydrochloride (mp 117–118°), and *N,N*-dimethyl-4-methoxy-β-hydroxyphenethylamine hydrochloride (mp 145°) involved a Houben–Hoesch condensation of anisole[1] with aminoacetonitrile hydrochloride[2], *N*-methylaminoacetonitrile hydrochloride[2], and *N,N*-dimethylaminoacetonitrile hydrochloride[2], respectively, followed by a sodium borohydride reduction of the ketone intermediate (9). The UV, IR, and NMR spectra verified the structure of the desired product in each case.

[1] Aldrich Chemical Co.
[2] K and K Laboratories.

Table I—Effects of Compounds in Prevention of Reserpine-Induced Hypothermia

Compound	Control[a]	Experimental[a]	Reference[a] (Iproniazid)
Unsubstituted	7.1 ± 0.6[b]	4.3 ± 0.9[b]	4.1 ± 1.3[b]
N-Methyl substituted	8.1 ± 0.7[b]	4.3 ± 0.7[b]	3.6 ± 0.7[b]
N,N-Dimethyl substituted	6.2 ± 0.5[b]	3.8 ± 1.3[b]	4.1 ± 1.3[b]

[a] Values represent changes in rectal temperature after 6 hr. [b] Mean ± standard error. Differences between control–experimental and control–reference groups were significant at $p \leq 0.05$. Controls received distilled water in a volume equal to that of the drug solution. All animals received reserpine (2.5 mg/kg ip) 1 hr after pretreatment. For details of treatment, see text.

Preliminary Studies—Initial screening for possible monoamine oxidase inhibition was done using the reserpine reversal technique of Pletscher (10). Male, Swiss–Webster albino mice, 21–54 g, were randomly divided into three groups of nine animals each for each study and were injected intraperitoneally as follows:

Group 1 (Control)—Distilled water (same volume as drug solution) 1 hr prior to injection of reserpine (2.5 mg/kg ip).

Group 2 (Experimental)—Phenethylamine derivative (200 mg/kg) 1 hr prior to injection of reserpine (2.5 mg/kg ip).

Group 3 (Reference)—Iproniazid (100 mg/kg) (reference compound) 1 hr prior to injection of reserpine (2.5 mg/kg ip).

Immediately after injection with reserpine, all animals were placed in individual plastic restraining housings. Rectal temperatures were monitored initially and at hourly intervals for 6 hr, using electronic thermometers[3].

Warburg Studies—As a further evaluation of possible monoamine oxidase inhibition, the effects of the compounds on the oxidation of tyramine hydrochloride[4] by rat brain mitochondria were investigated. Brain mitochondria (containing monoamine oxidase) were prepared from albino Wistar female rats, 150–200 g, according to the method of Brody and Bain (11), and monoamine oxidase activity was determined in the presence and absence of the respective compounds by conventional manometric techniques (12)[5].

In the manometric determinations, the main compartment of the Warburg flasks contained the compound studied (or buffer), 1 ml of mitochondrial suspension (representing 500 mg wet weight of original brain tissue), and sufficient 0.01 *M* phosphate buffer (pH 7.4) to make a total volume of 2.7 ml. The side arm of each flask contained 0.3 ml of 0.1 *M* tyramine hydrochloride. After the flasks were allowed to equilibrate for 15 min at 37°, the manometer valves were closed and the side arm contents of each were tipped in.

Manometers were set at 150 mm, and readings were taken at 15-min intervals for 90 min. Values for the 90-min reaction period were obtained for each flask and were multiplied by the respective flask constants after adjusting for changes in the thermobarometer. The total microliters of oxygen uptake for each flask was thus obtained.

Statistical Analysis—The mean ± standard error was determined for each control or treated group. Values thus obtained were compared, using the difference between the means and the standard error of the difference between the means for the respective control-treated pairs of groups. The Student *t* test was applied to the results, and the probability was determined.

[3] Telethermometer model 43TA with probes, model 402, Yellow Springs Instrument Co.
[4] Eastman Kodak Co.
[5] Precision Warburg apparatus, Precision Scientific Co.

Table II—Effects of Compounds on Oxidation of Tyramine Hydrochloride by Rat Brain Mitochondria

Compound	Concentration, *M*	Control[a]	Experimental[a]	Inhibition, %	*t* Value[b]
Unsubstituted	0.003	35.0 ± 1.6	28.5 ± 2.0	19	2.5
	0.006	42.6 ± 1.6	29.5 ± 1.2	31	6.6
	0.012	25.7 ± 1.1	12.0 ± 1.0	53	9.5
	0.024	40.6 ± 1.5	16.5 ± 0.8	59	14.8
N-Methyl substituted	0.003	48.9 ± 3.4	31.9 ± 2.5	35	4.0
	0.006	44.9 ± 3.0	26.0 ± 2.2	42	5.0
	0.012	60.6 ± 2.5	27.9 ± 1.5	54	11.3
	0.024	77.2 ± 5.0	23.5 ± 1.3	70	10.5
N,N-Dimethyl substituted	0.013	30.4 ± 1.4	24.8 ± 1.5	18	2.7
	0.025	27.5 ± 2.0	20.2 ± 1.1	27	3.2
	0.050	32.0 ± 1.7	20.5 ± 1.4	36	5.2
	0.100	33.3 ± 2.3	13.3 ± 1.2	61	7.7

[a] Each value represents the microliters of oxygen uptake (mean ± standard error) for 12 flasks. [b] All differences between means were significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

In studies of reserpine reversal (prevention or partial prevention of reserpine-induced hypothermia), the compounds produced significant ($p \leq 0.05$) effects at a dose of 200 mg/kg (Table I). Iproniazid, a known monoamine oxidase inhibitor, also partially prevented reserpine-induced hypothermia at a dose of 100 mg/kg ($p \leq 0.05$). While these results are not conclusive proof of monoamine oxidase inhibition by the compounds, they do suggest the possibility, since monoamine oxidase inhibitors usually exhibit the reserpine-reversal phenomenon (10).

Direct measurements of inhibition using Warburg studies showed that all three compounds inhibited monoamine oxidase in the concentration ranges employed (Table II). (±)-4-Methoxy-β-hydroxyphenethylamine hydrochloride, in concentrations of 0.003–0.024 *M*, produced significant ($p \leq 0.05$) inhibition of monoamine oxidase when compared to controls. Likewise, (±)-*N*-methyl-4-methoxy-β-hydroxyphenethylamine hydrochloride, in concentrations of 0.003–0.024 *M*, and (±)-*N,N*-dimethyl-4-methoxy-β-hydroxyphenethylamine hydrochloride, in concentrations of 0.013–0.1 *M*, also significantly inhibited monoamine oxidase.

A number of compounds possessing a phenethylamine nucleus are capable of inhibiting monoamine oxidase (13). The phenyl and nitrogen moieties of these compounds are usually unsubstituted. Phenylethanolamine derivatives exhibiting monoamine oxidase inhibition have all been unsubstituted (14). All *para*-methoxylated phenylethanolamines examined in this study inhibited monoamine oxidase. The fact that the secondary amine exhibited the greatest activity, followed by the primary amine, supports previous data. Based on available information, further speculation dealing with competition for the site of action on the enzyme and reversibility of the binding at this site would be difficult since these parameters are dependent on the particular substrate and tissue examined.

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