THE EFFECT OF SOME MONOAMINE OXIDASE INHIBITORS ON AMINE EXCRETION IN THE RAT

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Received April 25, 1960

This study was concerned with the effect of four monoamine oxidase inhibitors, pheniprazine (1-phenyl-2-hydrazinopropane, JB516), tranylcypromine (2-phenylcyclopropylamine), iproniazid (isopropyl-isonicotinyl hydrazine) and harmaline on the urinary excretion of six organic bases by the rat (methylamine, dimethylamine, pyrrolidine, piperidine, β-phenylethylamine and tryptamine). It has already been shown that administration of iproniazid or JB516 to humans results in a 3 to 6 fold increase in the level of urinary tryptamine (Sjoerdsma et. al., 1959). In guinea pigs treated with 150 mg./kg. of iproniazid, urinary excretion of tryptamine rose from less than 0.6 μg/day to 10 μg/day which was further increased to 83.0 μg/day by prior administration of 800 mg./kg. of L-tryptophan (Hess et. al., 1959). Jepson et. al. (1960) have demonstrated a rise in urinary β-phenylethylamine excretion both in normal and in phenylketonuric humans treated with monoamine oxidase (MAO) inhibitors. This increase in the phenylketonurics was 50 times greater than it was in normal subjects.

Materials and Methods

Groups of 6 white male Wistar rats were placed in stainless steel metabolism cages (2 per cage), and received Wayne Lab. Blox and water ad libitum. Urine from the animals was collected in test tubes attached to the outlets of the cages by a perforated rubber stopper. One ml. of 2 M HCl and 2 ml. of benzene were placed in the bottom of each tube to prevent loss of volatile

amines and retard bacterial growth. Urine collected after 24 and 48 hours was taken separately and pooled. The rats in each group were injected intraperitoneally with the drugs, the first injection being followed by a second 24 hours later. Total doses were as follows: iproniazid, 8 mg./rat; JB516, 4 mg.; tranylcypromine, 4 mg.; harmaline, 8 mg.[1].

The pooled urine from each group of 6 rats was adjusted to pH < 11.0, shaken with 2 vols. of benzene and the benzene extracted with 2 ml. 0.02 M HCl. The acid extract was shell frozen in 15 ml. centrifuge tubes, lyophilized and the solids reconstituted in 0.2 to 0.4 ml. of water. As recoveries of methylamine and dimethylamine obtained by this extraction were very low (2-3%), these two components were recovered by passing a stream of nitrogen through strongly basic urine into 0.1 M H_2SO_4 for 90 minutes. Methylamine and dimethylamine were liberated quantitatively from the urine and trapped in the acid. The acid was chromatographed without further concentration.

Separation of the components was effected by descending chromatography on Whatman #1 paper for 16 hours using two systems (BuOH, H_2O, HOAc, 5:4:1 and methylethylketone, t-butanol, water, acetic acid, 8:8:4:1). Sheets were air dried, dipped in 0.2% ninhydrin in acetone with 10% of glacial acetic acid and heated for 4 minutes at 110°C., shown to be optimal for maximum color development Areas corresponding to the amines were cut out, eluted with 75% acetone buffered at pH 8.0 by means of 1 ml. 1 M phosphate buffer per liter. The optical density of the eluate was determined in a spectrophotometer at 570 mμ except for pyrrolidine which was read at 360 mμ. Tryptamine, transformed to a β-carboline by this treatment (Jepson, 1953), was eluted with acetone to which was added 1/40 volume 0.02 M HCl. Its level was determined fluorometrically with an Aminco-Bowman spectrofluorometer (activating wavelength 490 mμ, fluorescent 540 mμ).

[1] JB516 was obtained through the courtesy of Lakeside Laboratories and tranylcypromine from Smith, Kline and French.

Results

Urine extracts chromatographed in the above systems separated into 10 components having characteristic R_f's, colors and fluorescence. Of these 10, 7 were identified having the following characteristics (R_f's refer to the system BuOH:H_2O:HOAc,:5:4:1): methylamine R_f .33, mauve, non-fluorescent; dimethylamine, R_f .40, mauve, non-fluorescent; ethylamine, R_f .46, blue, non-fluorescent;

Table 1

THE INFLUENCE OF MAO INHIBITORS ON URINARY EXCRETION OF AMINES BY THE RAT. Expressed as percent of control (mean value of 3 or 4 experiments, upper and lower values in parentheses)

Drug	Methyl-amine	Dimethyl-amine	Pyrrolidine	Piperidine	β-Phenylethyl-amine
JB516 (2 mg./rat/day)	350 (280,455)	103 (96,105)	66 (47,82)	73 (46,81)	503 (440,540)
Iproniazid (4 mg./rat/day)	316 (280,380)	113 (80,150)	110 (88,170)	102 (75,160)	1030 (740,1480)
Tranylcypromine (4 mg./rat/day)	53 (33,75)	64 (51,75)	45 (26,65)	24 (16,33)	367 (168,567)
Harmaline (4 mg./rat/day)	36 (18,60)	88 (72,91)	58 (56,64)	66 (37,88)	Masked by Harmaline

Table 2

EXCRETION OF TRYPTAMINE (μg/rat/day) FROM RATS INJECTED WITH VARIOUS MAO INHIBITORS IN THE FIRST 48 HOURS' TREATMENT (FOUR EXPERIMENTS)

Control	JB516	Iproniazid	Tranylcypromine	Harmaline
0.435 (0.31, 0.56)	3.35 (3.08, 4.50)	3.63 (2.56, 4.55)	4.08*	Masked by the drug

*Dose:2 mg./rat/day. One experiment only.

pyrrolidine, R_f .51, orange fluorescence; piperidine, R_f .59, violet, red fluorescence; tryptamine, R_f .68, faint purple, yellow-green fluorescence; β-phenylethylamine, R_f .77, blue, non-fluorescent.

The levels of these components in the urine of untreated rats as determined by the above semi-quantitative methods were (in μg excreted per rat per day): methylamine, 80; dimethylamine, 600; pyrrolidine, 220; piperidine, 350; tryptamine, < 0.7; β-phenylethylamine, 85.

The changes produced by the administration of MAO inhibitors on the levels of these components in the urine are shown in Table I, values being expressed as percent of that of the control. Tryptamine values are separately shown in terms of μg excreted per rat per day (Table II).

Discussion

The two MAO inhibitors, iproniazid and JB516, produced large increases in the urinary excretion of methylamine, tryptamine and β-phenylethylamine. Tranylcypromine affected the excretion of tryptamine and β-phenylethylamine but did not alter significantly the excretion of methylamine.

There are now at least 5 drugs which have been marketed as anti-depressants, all of which are hydrazines or substituted hydrazids. A number of other classes of compounds have been shown to inhibit MAO (Davison, 1958) but they appear to lack the anti-depressant activity characteristic of the 5 hydrazids. The effect of iproniazid and JB516 on the metabolism of methylamine may be related, therefore, to their activity as anti-depressants.

Acknowledgment: We wish to acknowledge the interest and advice of Dr. Paul H. Bell

References

Davison, A. N., Physiol. Rev. 38, 729 (1958).

Hess, S. M., Redfield, B. G. and Udenfriend, S., J. Pharmacol. and Exptl. Therap. 127, 178 (1959).

Jepson, J. B., Lovenberg, W., Zaltman, J. A., Oates, A., Sjoerdsma, A. and Udenfriend, S., Biochem. J. 74, 5p (1960).

Jepson, J. B. and Stevens, B. J., Nature 172, 772 (1953).

Sjoerdsma, A., Oates, J. A., Zaltman, P. and Udenfriend, S., J. Pharmacol. and Exptl. Therap. 126, 217 (1959).