Biochemical Pharmacology, 1961, Vol. 7, pp. 181–186. Pergamon Press Ltd., Printed in Great Britain.

EFFECT OF L-DOPA AND N-METHYL-N-BENZYL-2-PROPYNYLAMINE.HCl ON DOPA, DOPAMINE, NOREPINEPHRINE, EPINEPHRINE AND SEROTONIN LEVELS IN MOUSE BRAIN

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(*Received* 27 *December* 1960)

Abstract—A procedure for the determination of DOPA, dopamine, norepinephrine, epinephrine and serotonin in a single tissue homogenate is given. N-Methyl-N-benzyl-2-propynylamine.HCl (A19120, MO-911) causes increased dopamine, norepinephrine and serotonin levels in mouse brain. Dopamine levels return to normal within 48 hr, at which time the levels of norepinephrine and serotonin are still elevated. L-DOPA causes increased DOPA, dopamine and norepinephrine concentrations, decreasing in this order. After MO-911 followed by L-DOPA, the increases in DOPA, dopamine and norepinephrine are greater than with either drug alone or the sum of these increases. At the time of sacrifice, after the administration of MO-911 and L-DOPA, the mice were markedly hyperactive, but the relative contribution of the increases in DOPA, dopamine, norepinephrine or serotonin to this activity could not be determined.

N-METHYL-N-BENZYL-2-PROPYNYLAMINE.HCl (A19120, MO-911) is a potent non-hydrazide monoamine oxidase (MAO) inhibitor.[1] A dose of 100 mg/kg intraperitoneally causes no behavioral changes in mice, but subsequent to the further intraperitoneal administration of 200 mg of DL-dihydroxyphenylalanine (DL-DOPA) per kg a marked increase in activity and aggressiveness is obtained.[2, 3] This dose of DL-DOPA alone does not cause a change of activity in control mice.[4]

Bertler and Rosengren[5] report increased activity correlated with increased brain dopamine levels in rabbits given 600 mg of DOPA per kg. Carlsson *et al.*[6] also found increased dopamine and norepinephrine levels in mouse brain 6 hr after monoamine oxidase inhibitors. This suggests that the activity of mice given an inhibitor of MAO followed by DOPA may be due to increases in dopamine or norepinephrine. The relative increases in these amines and serotonin may be different after a MAO-inhibitor alone, and the duration of the increases may also differ. To investigate the changes in the concentration of these and related compounds, a new separation procedure for the determination of DOPA, dopamine, norepinephrine, epinephrine and serotonin in a single brain homogenate was developed and is included as part of this report.

METHODS

MO-911 dissolved in saline and L-DOPA (Calif. Corp. Biochem. Res., CfP grade) suspended in 0·5% Methocel were injected intraperitoneally, 0·02 ml/g body weight.

The concentration of MO-911 was corrected for HCl so that all doses refer to free base. White male mice in the weight range from 19 to 28 g were used.

Three pooled mouse brains were homogenized in 2 vols. of 0·01 N HCl and extracted by the method of Shore and Olin[7], using 3 ml of homogenate, 2 g of NaCl and 25 ml of *n*-butanol reagent. The second extraction was from 20 ml of the *n*-butanol with 20 ml *n*-heptane into 3 ml of 0·01 N HCl. In each extraction the tubes were shaken at 250 counts/min for 5 min. Aliquots of 0·6 ml of this final acid extract were analyzed for norepinephrine and epinephrine by using pH 5 and pH 3 buffer.[7] Fluorescence was measured in an Aminco spectrophotofluorometer at the uncorrected wavelengths of 397 mμ and 508 mμ.

Of the acid extract, 1·0 ml was placed on a water-washed Dowex 50W-x4 (200–400 mesh) Na-form column, 2 mm high and 5 mm wide. The sample was washed on with a single application of 2 ml of water. The DOPA was eluted in one tube with 8 ml of 0·025 M sodium phosphate buffer, pH 6·5, and the dopamine was eluted in a second tube with 8 ml of the same buffer containing 1 M KCl. This separation of DOPA and dopamine differs from that given by Bertler *et al.*[8] in that the acid extract is placed directly on the column without neutralization and elution is performed at the pH necessary for the development of fluorescence (pH 6·5), rather than with 1 N HCl; thus, two pH adjustments are eliminated. The procedure of Carlsson and Waldeck[9] was used to determine DOPA and dopamine concentrations in the eluates. Because fluorescent intensity developed slowly in these samples unless they were irradiated,[9] fluorescence was determined 22 hr later at the uncorrected activation and fluorescent wavelengths of 334 mμ and 380 mμ, respectively.

The remainder of the acid extract was used for analysis of serotonin by measuring fluorescence of the extract directly at the uncorrected wavelengths of 291 mμ and 342 mμ. It was unnecessary to adjust the acid concentration to 3 N[10] to obtain adequate sensitivity relative to the blank. The reading in 0·01 N HCl at the above wavelengths was thirteen times as sensitive as the reading at the peak at 545 mμ in 3 N HCl.

An acid blank was run with each experiment. Standard solutions of L-DOPA, dopamine, norepinephrine, epinephrine and serotonin in 0·01 N HCl were added in volumes of 0·01 to 0·04 ml to 3-ml aliquots of brain homogenate and a 3-ml aliquot of the same homogenate was analyzed as a control. The corrected fluorescent intensity (meter multiplier $\times$ $\%T$ corrected for that of the blank) of all compounds was linear in the concentration range reported. Standard solutions of dopamine, norepinephrine and serotonin in 0·01 N HCl were carried through the final color development with each set of samples analyzed.

The specificities of the procedures for each amine are not all absolute, and the contributions to the readings in each procedure due to the presence of other amines are given in Table 1. These contributions were determined by analyzing standard solutions containing one of the amines, 10 μg of free base per 3 ml of 0·01 N HCl, by the complete procedure, including extraction. Contributions of less than 1 per cent are not significantly different from blank values. The data in Table 1 show that the only significant contribution to the DOPA procedure is from dopamine, probably arising from leakage of the dopamine from the column during DOPA elution. The dopamine procedure is not influenced by large excesses of any of the other amines. Norepinephrine determinations are predominantly influenced by dopamine and epinephrine, the dopamine being the greater factor due to its greater concentration

in control and treated brain. The correction of the number of micrograms of norepinephrine by 2·4 per cent of the micrograms of DOPA present is not necessary in mouse brain, unless DOPA has been administered. Epinephrine is determined with only a slight contribution from norepinephrine. The serotonin procedure involves significant contributions to the fluorescence due to dopamine, norepinephrine and epinephrine. However, the quantity of epinephrine present in mouse brain is so small that the correction can be ignored, and the corrections for dopamine and norepinephrine are considerable only when concentrations of these amines are elevated.

TABLE 1. SPECIFICITY OF THE PROCEDURES FOR DETERMINATION OF DOPA, DOPAMINE, NOREPINEPHRINE, EPINEPHRINE AND SEROTONIN

Amine	Contributions of amines to the readings in each procedure, given as per cent of the weight of free base of contributing amine present*				
	DOPA procedure	Dopamine procedure	Nor-epinephrine procedure	Epinephrine procedure	Serotonin procedure
DOPA	100·0	0·8	2·4	0·6	0·8
Dopamine	5·8	100·0	8·0	0.0	3·0
Norepinephrine	−0·7	−0·3	100·0	1·4	2·6
Epinephrine	−0·2	0·1	57·0	100·0	2·8
Serotonin	−0·3	0·3	0·4	0·3	100·0

* Each value represents the mean of from two to six determinations.

The contributions of interfering amines listed in Table 1 must be made by calculating the correction on the basis of micrograms of interfering amine present. Thus, the norepinephrine should be corrected for dopamine and epinephrine before its level is used in correcting serotonin. Further correction of serotonin and DOPA for dopamine completes all the necessary corrections. The data have also been analyzed by solving five simultaneous equations on an IBM 1620 digital computer, using all the correction factors listed in Table 1. The values for micrograms of amine per gram of tissue obtained by the two methods of calculation do not differ appreciably.

RESULTS AND DISCUSSION

The concentrations of DOPA, dopamine (DA), norepinephrine (NE), epinephrine (EP) and serotonin in brain of control mice and mice treated with 200 mg of L-DOPA/kg 30 min before sacrifice, or with 100 mg MO-911/kg 24 or 48 hr before sacrifice, or with both drugs at the same dose and time before sacrifice, as was used for each drug alone (24 hr for MO-911), are given in Table 2. No changes in behavior were noted in the mice given MO-911. The mice which received only L-DOPA exhibited slightly increased activity when handled, but no changes were observed otherwise. The mice given MO-911 followed by L-DOPA were hyperactive, irritable and aggressive at the time of sacrifice.

The changes in DOPA, DA, NE and EP mouse brain concentrations during the first 24 hr after administration of 100 mg of MO-911/kg intraperiteoneally are shown in Fig. 1. Each value in Fig. 1 is the average of seven determinations, except for some control and 24-hr values, as given in Table 2.

TABLE 2. CONCENTRATION OF DOPA, DOPAMINE, NOREPINEPHRINE, EPINEPHRINE AND SEROTONIN IN WHOLE MOUSE BRAIN

Treatment	Activity	Mouse brain conc. (μg/g $\pm$ s.d.) (no. of detns.)				
		DOPA	Dopamine	Norepinephrine	Epinephrine	Serotonin
Controls	normal	0·08 $\pm$ 0·09 (12)	0·87 $\pm$ 0·16 (12)	0·36 $\pm$ 0·05 (10)	0·04 $\pm$ 0·01 (13)	0·82 $\pm$ 0·02 (3)
MO-911, 100 mg/kg, −24 hr	normal	0·11 $\pm$ 0·05 (7)	1·03 $\pm$ 0·07 (7)	0·62 $\pm$ 0·05 (6)	0·04 $\pm$ 0·01 (7)	1·14 $\pm$ 0·10 (8)
MO-911, 100 mg/kg, −48 hr	normal	0·09 $\pm$ 0·06 (7)	0·83 $\pm$ 0·03 (7)	0·55 $\pm$ 0·02 (7)	0·03 $\pm$ 0·01 (7)	1·16 $\pm$ 0·03 (7)
L-DOPA, 200 mg/kg, −30 min	slight hyperactivity	0·75 $\pm$ 0·24 (7)	2·80 $\pm$ 0·52 (7)	0·47 $\pm$ 0·06 (7)	0·04 $\pm$ 0·02 (7)	0·72 (2)
MO-911, 100 mg/kg, −24 hr L-DOPA, 200 mg/kg, −30 min	marked irritability	4·38 $\pm$ 0·44 (7)	9·47 $\pm$ 0·60 (7)	0·81 $\pm$ 0·15 (6)	0·04 $\pm$ 0·02 (7)	1·42 $\pm$ 0·05 (3)

The increase in the levels of dopamine, norepinephrine and serotonin in the brain after hydrazide type MAO-inhibitors is well documented, and also occurs with MO-911, an MAO-inhibitor of the propynylamine type (Table 2, Fig. 1). The concentration of DOPA in the brain at any time during the first 48 hr after MO-911 administration is not significantly higher than the control level. The epinephrine level is stable during the 48-hr period following administration of MO-911. Although epinephrine may be a substrate for MAO, adequate metabolic pathways for the metabolism of epinephrine other than that through MAO may be utilized.

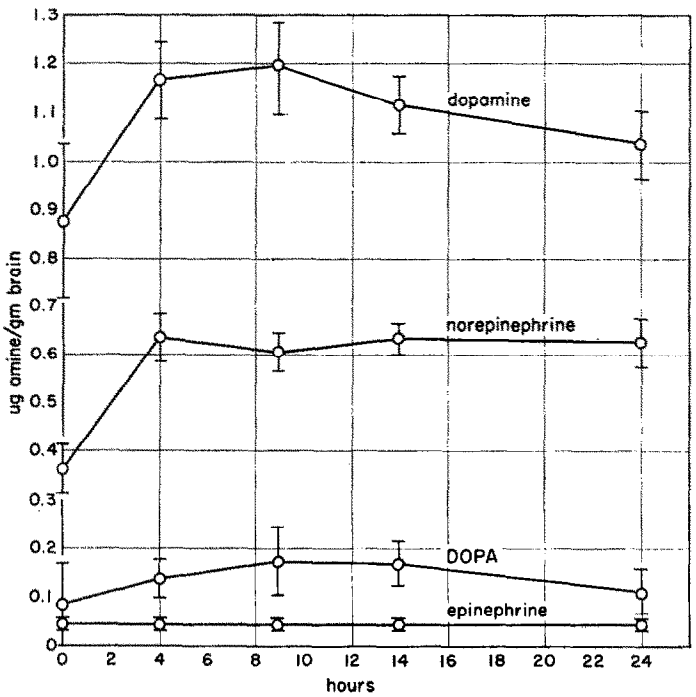

FIG. 1. Concentrations (together with their standard deviations) of DOPA, dopamine, norepinephrine and epinephrine in mouse brain following 100 mg of MO-911 (as free base) per kg intraperitoneally.

The duration of the increases in the levels of DA, NE and serotonin after MO-911, shown in Fig. 1 and Table 2, indicate that the amines return to control levels at different rates. Levels of dopamine in the brain reach a peak at about 9 hr (37 per cent above control) and decrease to control values by 48 hr. Norepinephrine concentrations in the brain are essentially stable in the period from 4 to 24 hr after MO-911 administration at a level about 72 per cent above control, and by 48 hr have decreased only slightly toward control values. This difference may be due to the relative rates of oxidative deamination of DA and NE by residual MAO activity, reported *in vitro* with a purified enzyme preparation[11] to be 100 for dopamine and 12 for norepinephrine. If these relative rates were operative *in vivo*, dopamine would fall to control levels before norepinephrine. Similarly relative rates of O-methylation could account for the result, but such rates with a purified O-methyltransferase system have been

reported by Axelrod and Tomchick[12] as essentially equal. The level of serotonin in the brain 24 and 48 hr after MO-911 remains about 40 per cent above control levels.

After administration of L-DOPA the percentage increase above control amine levels at 30 min is greatest for DOPA, and decreases in the order of metabolic conversion—DOPA, DA, NE and EP. Prior administration of the MAO-inhibitor MO-911 causes the percentage increases of DOPA, DA and NE to be much greater than after administration of L-DOPA alone, but the percentage increase is still greatest for DOPA and decreases in the order DOPA, DA, NE and EP. The failure of the epinephrine concentration to increase 30 min after the administration of L-DOPA, or of MO-911 and L-DOPA, may be due to the series of metabolic transformations prior to epinephrine formation. This is reflected in Table 2, in which it is shown that 30 min after the administration of L-DOPA, DOPA has increased over its control level by a factor of 9·4 and dopamine by a factor of 3·2, while norepinephrine is only 1·3 times its control level. These ratios after MO-911 and L-DOPA are DOPA 55, DA 11, and NE 2·2.

Increased levels of DOPA, dopamine, norepinephrine and serotonin in brain were present at the time of sacrifice of the mice exhibiting marked hyperactivity. The contribution of the increase in any one of these amines to the hyperactivity of the mice could not be determined. The slight increase in motor activity observed after administration of L-DOPA alone, however, occurred when only DOPA and dopamine levels were increased above those observed in animals showing no behavioral change.

In contrast to the changes in amines following L-DOPA, the effect of MO-911 is to elevate the levels of norepinephrine and serotonin for longer than 48 hr, while dopamine levels increase less and return to normal within 48 hr. This suggests that any effects associated with the long duration of monoamine oxidase inhibition produced *in vivo* by MO-911[1] may not be due to dopamine, but rather to norepinephrine or serotonin.

REFERENCES

1. J. D. TAYLOR, A. A. WYKES, Y. C. GLADISH and W. B. MARTIN, *Nature, Lond.* **187**, 941 (1960).
2. J. D. TAYLOR, A. A. WYKES, Y. C. GLADISH, W. B. MARTIN and G. M. EVERETT, *Fed. Proc.* **19**, 278 (1960).
3. G. M. EVERETT, *Psychopharmacology—Proceedings of the Second Meeting of the Inter-National Collegium of Neuro-Psychopharmacology, Basle, July* 1960. In press.
4. G. M. EVERETT, J. C. DAVIN and J. E. P. TOMAN, *Fed. Proc.* **18**, 613 (1959).
5. A. BERTLER and E. ROSENGREN, *Experientia* **15**, 382 (1959).
6. A. CARLSSON, M. LINDQVIST and T. MAGNUSSON, *J. Soc. Cien. Med. de Lisboa* **123**, 96 (1959).
7. P. A. SHORE and J. S. OLIN, *J. Pharmacol.* **122**, 295 (1958).
8. A. BERTLER, A. CARLSSON and E. ROSENGREN, *Acta Physiol. Scand.* **44**, 273 (1958).
9. A. CARLSSON and B. WALDECK, *Acta Physiol. Scand.* **44**, 293 (1958).
10. S. UDENFRIEND, H. WEISSBACH and D. F. BOGDANSKI, *Science* **122**, 972 (1955).
11. L. C. LEEPER, H. WEISSBACH and S. UDENFRIEND, *Arch. Biochem. Biophys.* **77**, 417 (1958).
12. J. AXELROD and R. TOMCHICK, *J. Biol. Chem.* **233**, 702 (1958).