Biochemical Pharmacology, Vol. 20, pp. 2299–2308. Pergamon Press, 1971. Printed in Great Britain

PHARMACOLOGICAL EFFECTS OF CARBARYL—I

THE EFFECT OF CARBARYL ON THE SYNTHESIS AND DEGRADATION OF CATECHOLAMINES IN THE RAT

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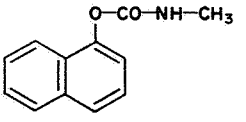
(*Received* 14 *October* 1970; *accepted* 29 *January* 1971)

Abstract—The pharmacological effects of 1-naphthyl *N*-methylcarbamate (carbaryl) in the rat were investigated with respect to catecholamine metabolism. Animals fed 700 ppm of carbaryl eliminated significantly higher amounts of urinary 3-methoxy-4-hydroxymandelic acid (VMA) as compared to control rats (over 300 per cent after 1 month). VMA excretion was maximal at about 30 days and then decreased, approaching normal values after 195 days. Dietary carbaryl fed at 100 ppm did not affect VMA excretion during 7 months of administration. A single oral carbaryl dose of 50 or 80 mg/kg resulted in a significant increase of urinary VMA, and the excretion of the corresponding alcohol, 3-methoxy-4-hydroxyphenylglycol (MHPG), also increased, but to a much lesser extent. Increased VMA excretion was also observed in adrenalectomized and hypophysectomized rats treated with carbaryl. Results showed a significant increase (68 per cent) in the turnover rate of norepinephrine (NE) in hearts from carbaryl-treated rats over that obtained in normal animals; but there was no change in the steady state concentration of the transmitter. It is suggested that the increase in synthesis rate of NE (and probably also the increased VMA excretion) is the result of increased sympatho-adrenergic activity, and that the adrenal medulla is not involved to any appreciable extent. The mechanism leading to increased nerve activity is presently unknown. Carbaryl strongly stimulated the pituitary–adrenocortical axis, as indicated by an increased corticosterone level in the plasma.

CARBARYL (1-naphthyl *N*-methylcarbamate), the structure of which is shown below, is an insecticide used effectively against a variety of insects which attack fruit trees and bean and cotton crops. It derives its toxic effects from being a potent cholinesterase inhibitor,[1] but, in general, its toxicity for mammals is of low order.[2] Although the biological fate of carbaryl has been extensively studied,[3–5] very little is known about its pharmacological effects in mammals.



The present investigation was initiated following the observation that repeated administration of a mixture of parathion, DDT and carbaryl induced an increased elimination of urinary 3-methoxy-4-hydroxymandelic acid (VMA) and 5-hydroxy-3-

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indolylacetic acid (5-HIAA) in the rabbit.[6] It was also postulated that an unknown mechanism was involved which resulted in an increased synthesis rate of the corresponding amines in nervous tissue. Through a series of experiments with individual pesticides, carbaryl was recognized as the active compound which is capable of producing changes in the metabolism of biogenic amines.

This study is an attempt to elucidate the mechanism involved in the changes of catecholamine metabolism produced by carbaryl administration.

METHODS

Male albino rats (Fischer strain 344) weighing 140–180 g (for long-term experiments) or 200–250 g (for short-term experiments) were used in this study. Carbaryl (technical grade, 99·9%) was obtained from Union Carbide Corp., New York, N.Y., and was administered in one of the following ways: (a) mixed with a standard diet of Purina laboratory chow at a concentration of 100 or 700 ppm and fed *ad lib.*, or (b) suspended in peanut oil and given orally at a single dose of 50, 80 or 250 mg/kg, or in three single doses of 80 mg/kg each for 3 successive days. Control rats received only untreated Purina laboratory chow or peanut oil.

Urinary catecholamines and their metabolites. Urine was collected over hydrochloric acid and catecholamines were determined by the method of von Euler and Lishajko.[7] Filtered urine samples were analyzed for 3-methoxy-4-hydroxymandelic acid (VMA) according to the method of Pisano *et al.*[8] and for the corresponding alcohol, 3-methoxy-4-hydroxyphenylglycol (MHPG), a major metabolite of catecholamines in the rat, by the method of Sapira.[9] Urinary creatinine was measured by the Jaffé reaction.[10]

Determination of the turnover rate of heart norepinephrine. Two hr after the rats were given a single oral dose of carbaryl (80 mg/kg), tracer doses (0·2 μg/kg) of racemic norepinephrine (NE-7-^{3}H), purchased from Amersham/Searle Corp. (specific activity, 7·47 c/m-mole), were injected intravenously. The rats were killed by decapitation at different intervals and the hearts were dissected, washed in saline, weighed and homogenized in 5% trichloroacetic acid with an ultra homogenizer. NE in the supernatant was determined fluorometrically according to the procedure of von Euler and Lishajko.[7] The mean recovery of NE was 76 per cent; all tissue values were corrected for recovery. For determination of the radioactivity in the sample, 0·5 ml of the acetic acid eluate obtained after alumina column chromatography was added to 15 ml of a scintillation solution (Butyl-PBD in dioxane) and counted with a Nuclear Chicago liquid scintillation spectrometer. Counting efficiency was determined by external standardization and ranged from 41 to 45 per cent.

The fractional turnover rate constant (K) was determined from the rate of NE-^{3}H decline. The values for NE-^{3}H were logarithmically transformed for calculation of the linearity of regression and standard error of the regression coefficients.[11] The turnover rate was calculated as the product of the steady state NE level and of K, the rate constant of NE-^{3}H decline.[12]

Adrenalectomy and hypophysectomy. The rats were anesthetized with Metofane, dorsolateral incisions were made, and bilateral adrenalectomies were performed. The animals were then maintained on 0·9% NCl and regular food for 7 days before the administration of carbaryl.

Hypophysectomized rats, weighing 200–250 g, were purchased from Hormone Assay Labs, Chicago, Ill. Carbaryl was administered to these rats on the tenth day after surgery.

Plasma corticosterone. The rats were decapitated and blood was collected in vessels containing heparin. The plasma was separated and, if corticosterone was not determined immediately, was frozen and stored at −10°. The concentration of corticosterone was estimated by the fluorescence method of Silber *et al.*,[13] using an Aminco–Bowman spectrophotofluorometer (excitation, 470 mμ; fluorescence, 503 mμ).

Tissue levels of carbaryl. Carbaryl was quantitatively determined spectrophotometrically in brain and heart using the diazotized sulfanilic acid reaction according to the method of Fargó,[14] and in blood by a modification of the same method involving no protein precipitation.

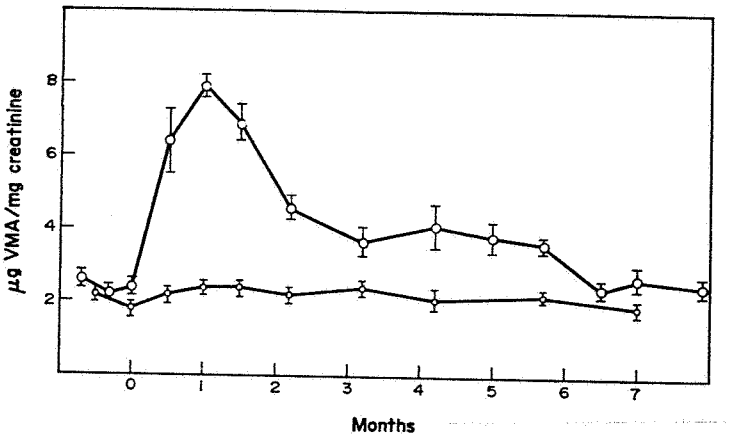


FIG. 1. Urinary VMA of rats fed 100 (small circles) and 700 ppm of carbaryl (large circles) for 8 months. Points represent mean values of five to seven determinations, each of a pooled urine sample from four rats. Bars indicate ± S.E. Urine samples were collected during 24 hr.

RESULTS

Urinary catecholamine metabolites. Figure 1 illustrates the excretion rate of urinary VMA in rats fed 100 and 700 ppm of carbaryl. Rats fed the low concentration did not show any significant increase in VMA excretion over a period of 7 months. The rats given 700 ppm eliminated higher amounts of VMA; at 30 days the amount had increased by over 300 per cent. During the following months, the elimination rate slowly declined and approached normal values after 195 days.

An increased elimination rate of VMA also occurred in rats given a single oral dose of carbaryl at 50, 80 or 250 mg/kg (Fig. 2). A dose–response relationship was suggested by the two lower doses; however, at 250 mg/kg a corresponding increase in VMA was not obtained. The rats given 80 mg/kg of carbaryl were allowed to recover for 20 days (urinary VMA had returned to normal) and were then given another single dose of carbaryl at 80 mg/kg. The magnitude and duration of the transient increase in urinary VMA resulting from the second dose corresponded closely with those of the first (Fig. 2).

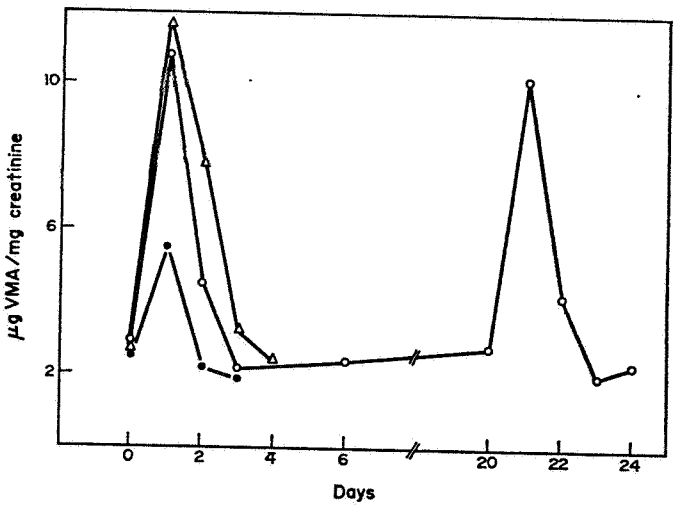


FIG. 2. Urinary VMA in rats given a single oral dose of carbaryl: (●) 50 mg/kg, (○) 80 mg/kg, (△) 250 mg/kg. Carbaryl was administered at 0 time and at 20 days. Points represent mean values of three determinations, each of a pooled urine sample from four rats. Urine samples were collected during 24 hr.

Figure 3 illustrates the elimination rate of urinary VMA and MHPG in rats treated with a single dose (80 mg/kg) or three successsive daily doses (80 mg/kg each) of carbaryl. In the latter group, the second and third doses did not produce a corresponding increase of VMA and the rats did not maintain the increased VMA level observed after the first dose. However, the time required for VMA values to return to normal was lengthened.

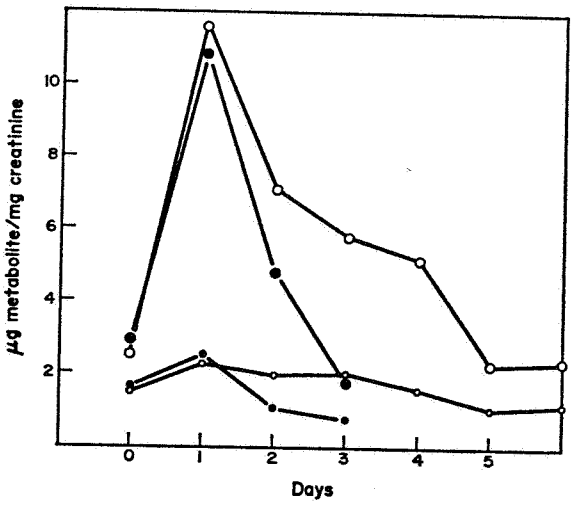


FIG. 3. Urinary VMA (large circles) and MHPG (small circles) in rats treated with a single carbaryl dose (solid circles) or three successive daily doses (open circles). Carbaryl (80 mg/kg) was administered at 0 time. Points represent mean values of three determinations, each of pooled urine sample from four rats. Urine samples were collected during 24 hr.

The MHPG values also increased, but to a much smaller extent than VMA. The MHPG also remained elevated somewhat longer in the rats given three doses of carbaryl than in those given a single dose. The amounts of unchanged catecholamines in the urine of treated rats were not significantly different from those of the control animals.

Adrenalectomy and hypophysectomy. Table 1 shows the effect of a single carbaryl dose on VMA excretion in adrenalectomized and hypophysectomized rats. VMA elimination showed the same pattern as in the intact animals; however, the baseline values for the "control" animals were higher than those for the intact control animals (Figs. 1–3).

TABLE 1. EFFECT OF CARBARYL ON URINARY VMA EXCRETION IN ADRENALECTOMIZED AND HYPOPHYSECTOMIZED RATS*

Period of urine collection (hr)	(μg VMA/mg creatinine ± S.E.)	
	Adrenalectomized†	Hypophysectomized‡
Control	3·8 ± 0·3	4·6 ± 0·5
0–24	11·0 ± 0·8	10·9 ± 1·3
24–48	5·0 ± 0·4	9·1 ± 0·7
48–72	4·1 ± 0·4	5·5 ± 0·4
72–96	3·5 ± 0·2	4·7 ± 0·3

* Carbaryl dose, 80 mg/kg.
† Mean of four determinations.
‡ Mean of six determinations.

Heart norepinephrine turnover. After pretreatment with carbaryl, the NE-^{3}H taken up by the heart declined with a half-life of 6·9 hr, compared to a control value of 12·2 hr (Fig. 4). Turnover rates, calculated from the data in Fig. 4, are given in Table 2. There was no significant difference in the steady state NE levels between the treated and control animals. However, the rate constants for NE increased in carbaryl-treated animals, showing a significant increase in turnover rate.

Plasma corticosterone. The results obtained from rats treated with a single dose of carbaryl are shown in Fig. 5. The corticosterone level was increased by about 125 per

TABLE 2. EFFECT OF CARBARYL ON TURNOVER RATE OF HEART NE

Treatment	N*	Level in heart (μg/g ± S.E.)	Rate constant of NE-^{3}H decline [K (hr^{-1}) ± S.E.]	Turnover rate† (μg/g/hr)	Increase in turnover rate (%)
Control	22	1·04 ± 0·05	0·057 ± 0·003	0·059	
Carbaryl	32	0·99 ± 0·04	0·100 ± 0·005	0·099	68

* N represents the total number of animals used.
† Turnover rate is product of steady state level and of K.

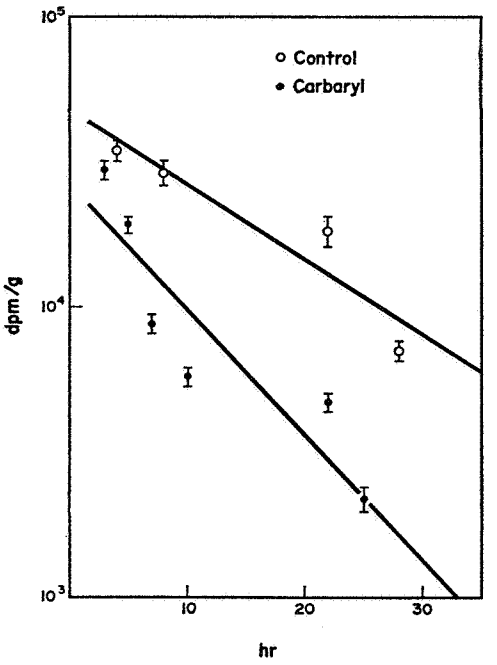


FIG. 4. Disappearance of NE-^{3}H from the rat heart after the i.v. administration of racemic NE-^{3}H (0·2 μg/kg). Each point represents the mean ± S.E. of five to six animals. The slope of the decline of NE-^{3}H was calculated by the method of least squares. Carbaryl (80 mg/kg) was given 2 hr before NE-^{3}H administration.

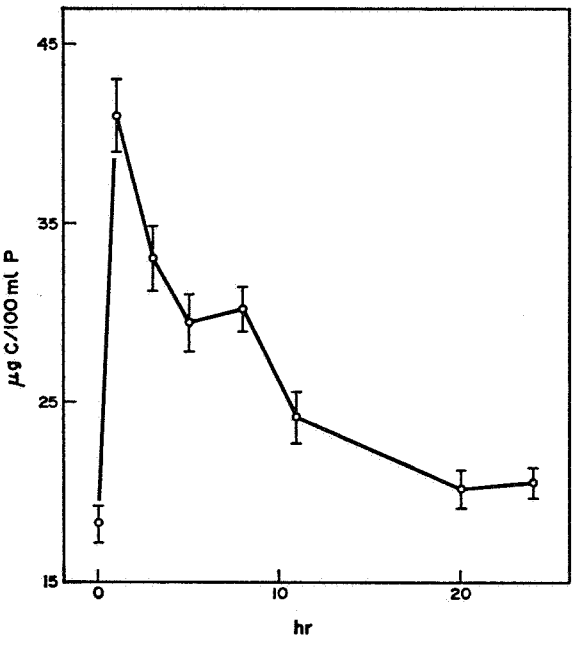


FIG. 5. Effect of a single carbaryl dose (80 mg/kg) on corticosterone (C) in plasma (P). Points represent means of at least five animals and bars indicate ± S.E.

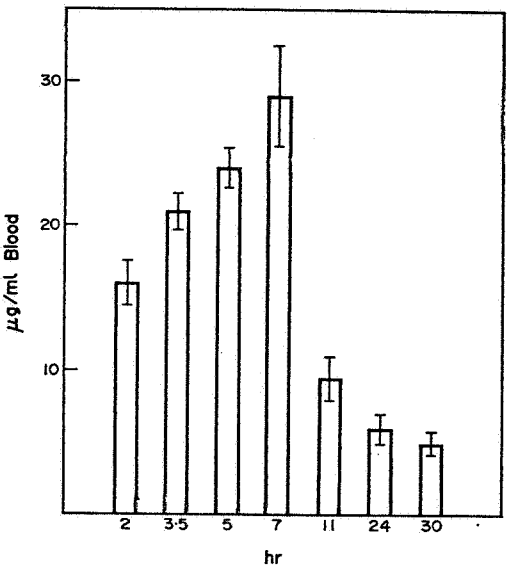


FIG. 6. Concentration of carbaryl in blood after a single oral dose (80 mg/kg). Data represent means of at least five animals and bars indicate ± S.E.

cent 1 hr after the oral administration of the carbamate, and probably represents the maximum elevation of the glucocorticoid. The level dropped thereafter and approached pretreatment levels after 20 hr. In control experiments, the administration of peanut oil alone did not cause an increase in the plasma corticosterone after 1 hr. The groups of rats fed 100 and 700 ppm of carbaryl did not show significant increases in plasma corticosterone after 90 days.

Tissue levels of carbaryl. Table 3 shows the concentrations of carbaryl in blood, heart and brain of rats fed carbaryl or given a single oral dose. The concentration in blood was also studied during 30 hr after a single oral dose (Fig. 6). The maximum concentration of carbaryl was probably reached after 7 hr.

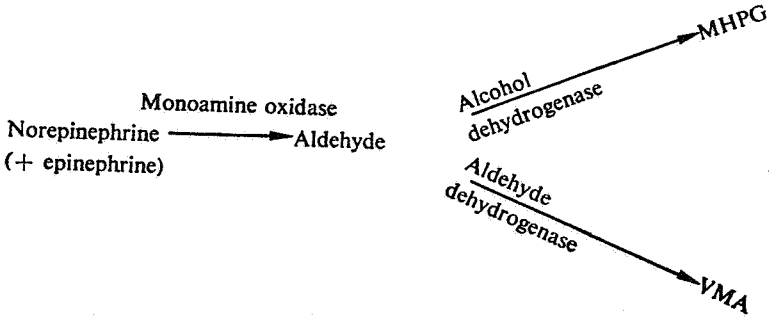
TABLE 3. TISSUE LEVELS OF CARBARYL

Treatment	Time after administration of carbaryl	Concentration of carbaryl		
		Whole blood (μg/ml ± S.E.)	Heart (μg/g ± S.E.)	Brain (μg/g ± S.E.)
Since dose (80 mg/kg)	2 hr	16·2 ± 1·5	2·60 ± 0·22	3·45 ± 0·24
Rats fed 700 ppm of carbaryl	90 days	4·8 ± 0·8	0·85 ± 0·10	0·68 ± 0·06
Rats fed 100 ppm of carbaryl	90 days	2·7 ± 0·8	<0·5	<0·5

DISCUSSION

The data show that the elimination rate of urinary VMA was significantly increased after carbaryl administration. After a single dose, the high excretion rate persisted for at least 2 days, but approached normal values on the third day. Doses of carbaryl higher than 80 mg/kg failed to produce a correspondingly greater increase of urinary VMA, suggesting that the response was maximal at this particular dose or that absorption from the gut was limited, or both. In favor of the theory of maximum response is the observation that the elevated response produced by the first dose was not maintained after the second and third daily doses (Fig. 3). The possibility of limited absorption of the carbamate (which is probably a consequence of poor solubility in oil) also gains support from the amount of carbaryl taken up by the organs. After 7 hr (Fig. 6), circulating carbaryl as well as its concentration in most tissues accounted for only 4–7 per cent of the administered dose.

According to Sapira,[9] the VMA values obtained by the method of Pisano *et al.*[8] represent the sum of VMA and MHPG, and the absolute values of VMA can be obtained only by subtracting the MHPG values from the total. Therefore, MHPG increased by about 50 per cent, whereas VMA increased by 540–930 per cent (Fig. 3). This suggests a selective effect on the pathways involved, as shown in the following simplified equation in which intermediates are omitted.



A plausible explanation is that the effect of carbaryl may involve preferential activation of the NAD- and NADP-linked aldehyde dehydrogenase over the alcohol dehydrogenase system. It is not known whether such activation involves induction of the enzyme or modification of the coenzymes ratio, or both. It is also probable that methyl transferase could become activated. Carbaryl-treated adrenalectomized and hypophysectomized rats also showed a significant increase in urinary VMA. The relatively high baseline values of the "control" (Table 1) may be explained by increased sympathetic activity.[15,16]

The increased excretion of VMA reflects an increased degradation rate (and synthesis rate) of catecholamines. Since adrenalectomy failed to influence the higher excretion rate of VMA in the treated animals, it is postulated that the increased synthesis observed in the present experiments is primarily the result of increased sympathoadrenergic activity. The increased turnover rate of heart NE is in accord with this postulation, and probably indicates an augmentation of synthesis, utilization and degradation of the amine. Directly or indirectly, the enzyme systems involved in

catecholamine metabolism are probably activated by carbaryl. The elevation of myocardial monoamine oxidase activity in response to increased adrenergic nerve activity after adrenalectomy is an interesting parallel.[15]

The mechanism leading to the increased synthesis of NE is not known. The present experiments indicate that the adrenal medulla does not contribute appreciably to this increase. Experiments with carbaryl, therefore, furnish additional evidence for the concept that the adrenal medulla and other parts of the sympathetic nervous system can be selectively and individually activated, not only by central control but also by chemicals.[17] The increased synthesis also appears to include a corticosterone-independent component and there is some evidence to suggest that the increase is also independent of the anticholinesterase action of the carbamate. This is provided by the observation that two anticholinesterase carbamate insecticides, Baygon (*o*-isopropoxyphenyl methylcarbamate) and Dimetilan [1-(dimethylcarbamoyl)-5-methyl-3-pyrazolyl dimethylcarbamate], administered at a single oral dose of 10 and 5 mg/kg, respectively, failed to influence the rate of urinary VMA elimination.

One of several explanations for the action of carbaryl is that it may directly affect the adrenergic nerve endings. Although carbaryl stimulates the adrenergic nerve activity and accelerates peripheral NE synthesis (with possible subsequent modification of the baroreceptors), there is also some evidence of increased turnover of NE in the central nervous system (A. Hassan, unpublished data).

The chronic administration of carbaryl at 700 ppm also increased the elimination of urinary VMA, particularly during the first 2 months (Fig. 1). By comparison with the events taking place after a single acute dose, it may be postulated that the higher elimination rate of VMA is also associated with an increased peripheral (and possibly central) sympatho-adrenergic activity. The near-normal values reached after 6 months, in spite of continuation of carbaryl intake, are an indication of adaptation which may be related to a faster carbaryl metabolism.

The administration of carbaryl strongly stimulates corticosterone secretion by the adrenals. Normally, corticosterone secretion would be considered an index of the release of ACTH. Figures 5 and 6 reveal no strict correlation between blood carbaryl and corticosterone levels, suggesting that a carbaryl concentration of less than 16·2 μg/ml in blood was sufficient to trigger a maximum corticosterone secretion.

In general, rats fed 100 and 700 ppm of carbaryl did not show apparent signs of toxicity. Only a very small number of the rats given acute oral doses (80 mg/kg) showed mild tremors within 2 hr and these rats quickly recovered. Although the toxicity of carbaryl may involve central effects, the toxicological implications of the changes in the metabolism of neurological amines are presently unknown. Pharmacological effects, however, include activation of the pituitary–adrenal axis and increased sympatho-adrenergic activity with concomitant increased VMA elimination.

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