The Effects of Pretreatment with Reserpine, α-Methyl-*p*-tyrosine, or Prostaglandin E_1 on Adrenergic Salivation (36553)

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In a previous paper (1), we described the effects of low ambient temperature on adrenergic salivation. These findings suggested an exploration of the effects of pretreatment with hypothermic drugs on responses to sialogogues. We chose three such drugs, reserpine, α-methyl-*p*-tyrosine (α-MT), and prostaglandin E_1 (PGE_1). Reserpine is known to deplete norepinephrine stores while α-MT inhibits its tyrosine hydroxylase activity, thereby blocking norepinephrine synthesis. The effects of PGE_1 on the sympathetic nervous system are less clear. Several investigators have reported, however, that PGE_1 has adrenergic blocking activity (2, 3).

Methods and Materials. Salivation of mice (male NIH, about 1 month old, weighing 16 to 22 g) was graded visually 0, 1, 2 and 3 as follows: 0, lips and jaws completely dry; 1, saliva spreading to lower lip; 2, saliva spreading to both upper and lower lips and jaws; 3, saliva spreading over the chest. Salivation for each mouse was recorded every 5 min for the first 30 min after the intraperitoneal injection of a sialogogue. The sum of the six successive gradings represented the salivation response, with a possible range of 0 to 18 (4).

Body temperature (T) was measured with a Telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). The thermocouple probe was inserted 3 cm into the rectum. Temperatures were recorded before treatment (T_0) and 15 and 30 min after the injection of the sialogogue. The temperature response (TR) was expressed as:

$$TR = 2\,\Delta T_{15} + \Delta T_{30}$$

As explained in the first paper of this series (4), the temperature response is an approximation of the area enclosed by the curve of temperature (T) plotted against time (t) from t_0 to t_{30} and the line, $T = T_0$, from $t = 0$ to $t = 30$. In some figures we have also included values of T_0 and the highest (T_{MAX}) or the lowest (T_{MIN}) observed temperatures.

Experiments were designed to compare all treatments in balanced randomized blocks of n (usually 12) mice. The desired number of replications for each treatment was obtained by increasing the number of blocks.

The data obtained from each experiment were subjected to an analysis of variance. When the same number of replicates were obtained for each treatment, the mean values were tested for statistically significant differences by Tukey's method of multiple comparisons (5). When the number of replicates were unequal, the treatment means were tested for significant differences by Scheffe's method of multiple comparisons (5).

Drugs were obtained from the following sources: *d*-amphetamine sulfate, K & K Labs., Plainview, NY; *l*-isoproterenol-d-bitartrate dihydrate, Sterling Winthrop Research Institute, Rensselaer, NY; *l*-norepinephrine bitartrate, Winthrop Labs., Special Chemical Dept., New York, NY; cocaine hydrochloride, Merck & Co., Inc., Rahway, NJ; reserpine phosphate, lyophilized, Ciba Pharmaceutical Co., Summit, NJ; the methyl ester hydrochloride of *dl*-methyl-*p*-tyrosine (H 44/68), A. B. Hassle, Goteborg, Sweden; prostaglandin E_1, the Upjohn Company, Kalamazoo, MI. Doses are expressed as milligrams of base per kilogram. All drugs were

injected intraperitoneally (ip).

Results. Effects of reserpine and α-MT on responses to agonists at room temperature and at elevated ambient temperatures. Mice tested 4 hr after reserpine (10 mg/kg, ip) did not salivate in response to *d*-amphetamine or *l*-norepinephrine after cocaine, but did salivate after *l*-isoproterenol (Fig. 1). The hypothermia caused by the reserpine is evident from the $T_{4\ hr}$ values obtained before administration of the adrenergic drugs. The temperature responses were not decreased by the reserpine treatment but the T_{MAX} values for *d*-amphetamine and *l*-norepinephrine after cocaine were still significantly lower than

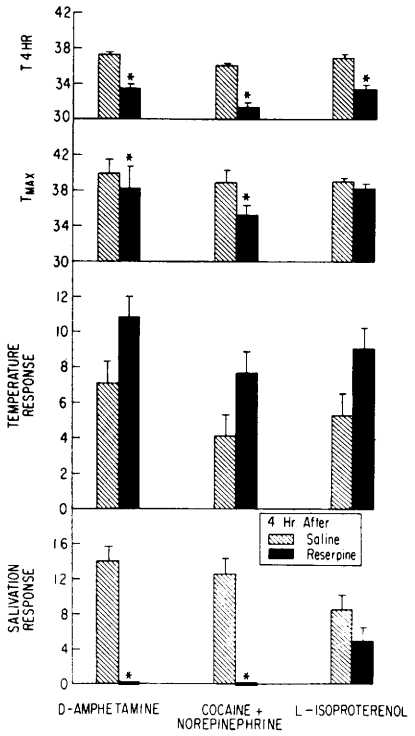

FIG. 1. The effect of pretreatment with reserpine (10 mg/kg, ip) on salivation and temperature responses to *d*-amphetamine (7.3 mg/kg), *l*-norepinephrine [0.5 mg/kg 5 min after cocaine (18 mg/kg)], and *l*-isoproterenol (300 mg/kg). Responses were tested 4 hr after administration of reserpine or saline. Each column represents the mean of values obtained on 6 mice ± SE. An asterisk means that the value for mice pretreated with reserpine differed significantly ($p < .05$) from that obtained on control mice which had been pretreated with saline.

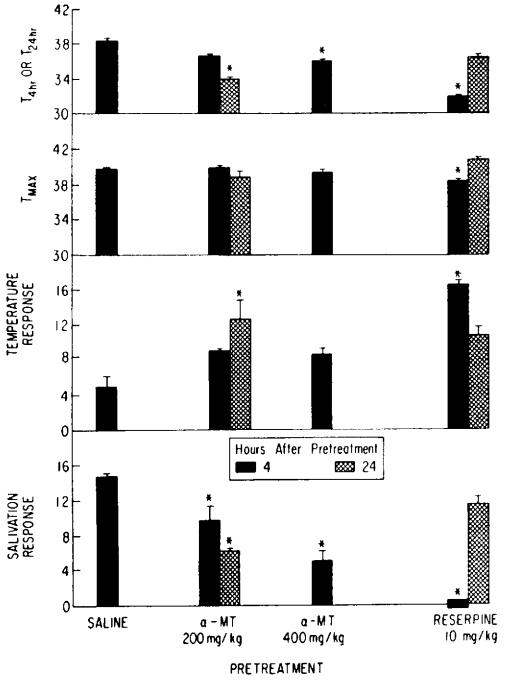

FIG. 2. Comparison of salivation and temperature responses to *d*-amphetamine (7.3 mg/kg) 4 hr after administration of saline (13 mice), α-MT (200 mg/kg in 6 mice and 400 mg/kg in 10 mice), and reserpine (10 mg/kg in 6 mice) and 24 hr after the smaller dose of α-MT (6 mice) and reserpine (6 mice). Responses were not tested 24 hr after the larger dose of α-MT because the mice which had been treated died before 24 hr. Each column represents the mean of values obtained on 6 mice + SE. An asterisk indicates that the response to *d*-amphetamine in mice pretreated with α-MT or reserpine differed significantly ($p < .05$) from that in mice pretreated with saline.

those obtained with animals pretreated with saline. T_{MAX} values for *l*-isoproterenol were about the same in mice pretreated with saline or reserpine.

Twenty-four hours after reserpine pretreatment, *d*-amphetamine produced salivation which was comparable to that in saline-pretreated mice (Fig. 2). In contrast, α-MT (200 mg/kg, ip) depressed salivation responses to *d*-amphetamine at least as effectively when administered 24 hr before *d*-amphetamine as at 4 hr. This compound, after a dose of 400 mg/kg, blocked at 4 hr approximately two-thirds of the salivation response to *d*-am-

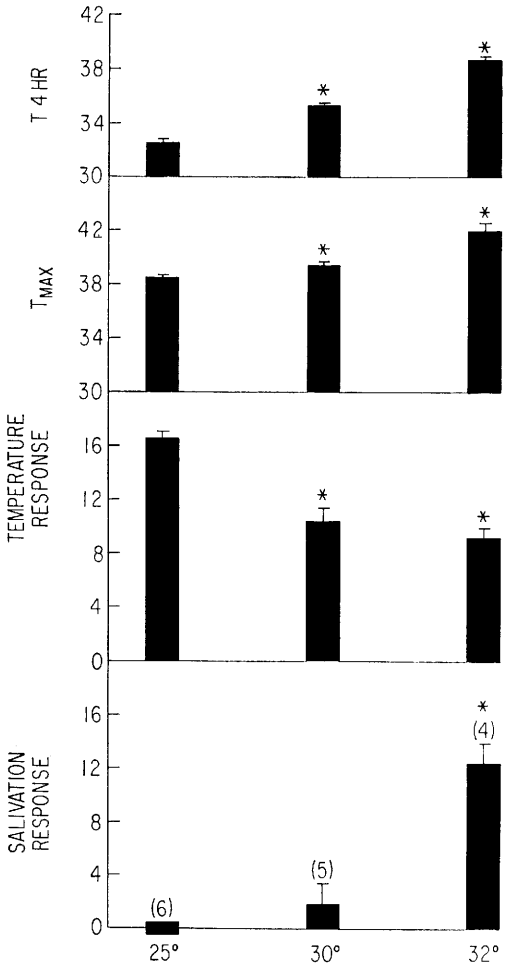

FIG. 3. The influence of elevated ambient temperature on responses to *d*-amphetamine (7.3 mg/kg) measured 4 hr after reserpine (10 mg/kg, ip). The responses are mean values ± SE of responses in the number of mice indicated in parentheses. An asterisk indicates that the responses differed significantly ($p < .05$) from those in mice tested at room temperature (25°). The mice were placed in a cage maintained at the indicated ambient temperature 3 hr after the administration of reserpine.

phetamine, despite unchanged T_{MAX} values (Fig. 2). The response to *d*-amphetamine could not be tested 24 hr after this dose of α-MT because all six mice died before 24 hr. As also shown in Fig. 2, α-MT lowered colonic temperature ($T_{4\ hr}$ or $T_{24\ hr}$) more at 24 hr than at 4 hr; the opposite was true of reserpine.

The suppression of salivation responses to d-amphetamine by reserpine was temperature dependent (Fig. 3). At room temperature (25°), no salivation occurred after an optimum dose of *d*-amphetamine. At 30°, slight salivation was observed while at 32°, the salivation responses to *d*-amphetamine were almost equal to those observed at room temperature in mice pretreated with saline. The colonic temperatures of mice pretreated with reserpine were significantly higher at these elevated ambient temperatures than at room temperature and were within the range recorded in untreated mice at room temperature (Fig. 3).

Effect of reserpine on heat-induced salivation and hyperthermia. Exposure to 40° for 30 min caused a salivation response of 7.3 ± 0.8 and a temperature response of 23.0 ± 1.4 in 6 mice tested 4 hr after treatment with reserpine (10 mg/kg). These values should be compared with a salivation response of 14.3 ± 1.2 and a temperature response of 7.4 ± 0.5 in 6 mice exposed to the same temperature 4 hr after an injection of saline. Peak temperatures of 42.7° were recorded for the reserpine-pretreated mice and 40.2° for the saline controls. Exposure to 40° was toxic to mice pretreated with reserpine. Although all six mice survived the experiment, five mice died soon afterward. Thus, reserpine decreased heat-induced salivation responses, but not as effectively as some other blocking agents (1).

Effects of prostaglandin E_1 (PGE_1) on salivation and temperature responses to adrenergic agonists. PGE_1 was a hypothermic drug when administered alone in doses of 0.3–1.0 mg/kg, ip (Figs. 4 and 5), as evident from the large negative temperature responses and the low T_{MIN} values measured after injections of saline in mice pretreated with PGE_1. Lower doses (0.05–0.10 mg/kg) did not modify body temperature significantly.

The effect of PGE_1 on salivation and temperature response to *d*-amphetamine is shown in Fig. 4. It is clear that PGE_1, in doses too small to produce hypothermia (0.05, 0.075 and 0.10 mg/kg) significantly depressed salivation. Larger doses of PGE_1 depressed both temperature and salivation responses to *d*-amphetamine.

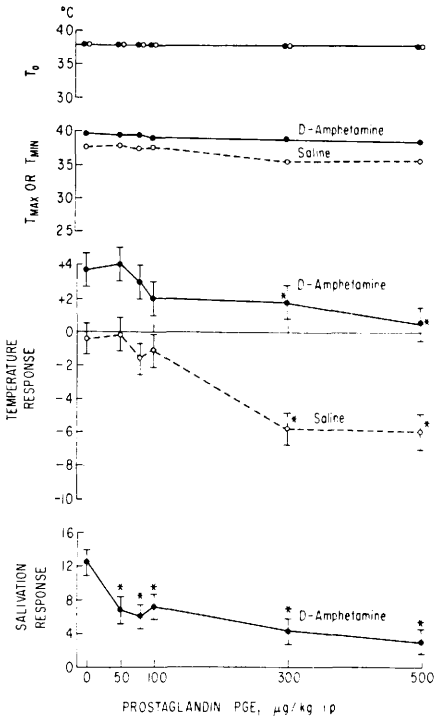

FIG. 4. The influence of PGE_1 on salivation and temperature responses to saline (○) or to *d*-amphetamine (7.3 mg/kg) (●), administered 5 min after PGE_1. The T_0 values represent the initial body temperatures in mice before receiving PGE_1. An asterisk indicates that the value differed significantly ($p < .05$) from the response in mice tested 5 min after an injection of saline (0 dose of PGE_1). Each point is the mean (± SE) of values obtained on 10 mice.

PGE_1, in doses of 0.5 and 1.0 mg/kg, although markedly hypothermic, failed to antagonize either the salivation or the temperature response to a combination of cocaine (18 mg/kg) and *l*-norepinephrine (0.5 mg/kg) (Fig. 5). This figure also shows that the dose of *l*-norepinephrine alone or cocaine alone did not cause salivation or hyperthermia, but reduced significantly the hypothermic effect of PGE_1. Relatively large doses of PGE_1 were required to reduce salivation and temperature responses to *l*-isoproterenol (Fig. 6). Even doses as high as 500 µg/kg (6 mice) and 1 mg/kg (4 mice) had no greater effects on salivation and temperature responses to *l*-isoproterenol than 300 µg/kg.

Discussion. Mice are relatively insensitive to both adrenergic and cholinergic sialogogues, as evident from the dose–response curves (4). Although large doses of either *l*-epinephrine alone or *l*-norepinephrine alone do produce salivation in the mouse (4), in this study we have used *l*-isoproterenol as a directly acting adrenergic amine with low toxicity and *d*-amphetamine as an indirectly acting amine. The experiments with *l*-norepinephrine after cocaine provide additional information about the cocaine effect.

It is surprising that salivation and temperature responses to adrenergic agonists in mice exposed to 4° (1) differed from those in mice tested 4 hr after the administration of reserpine, since reserpine pretreatment lowered body temperature to a far greater degree than exposure to cold. In the cold room, all adrenergic salivation was virtually abolished and the temperature responses to adrenergic agonists were reversed. In contrast, reserpine pretreatment 4 hr before the administration

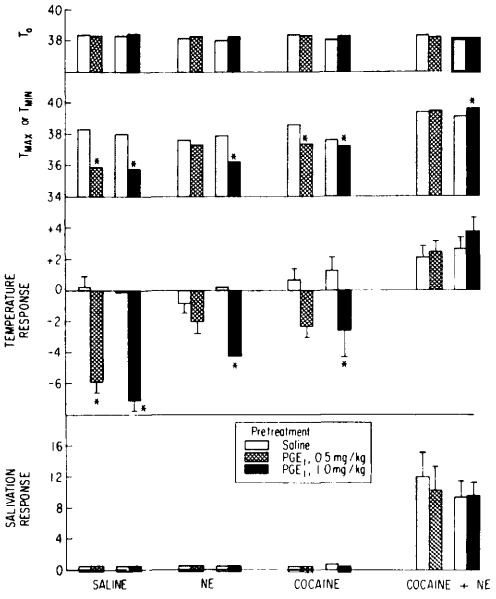

FIG. 5. The effect of PGE_1 (0.5 and 1.0 mg/kg) on salivation and temperature responses to saline, *l*-norepinephrine (0.5 mg/kg), cocaine (18 mg/kg) and *l*-norepinephrine 5 min after cocaine. Each value is the mean + SE of measurements in 8 mice. An asterisk indicates that the value differed significantly ($p < .05$) from that in mice pretreated with saline.

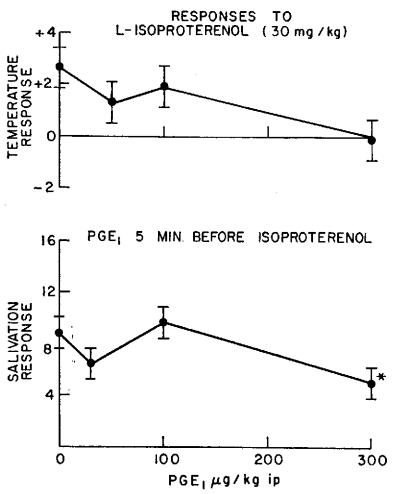

FIG. 6. The effect of administration of PGE_1 5 min before the sialogogue on salivation and temperature responses to *l*-isoproterenol (30 mg/kg ip). Each point is the mean ± SE of responses in 9 mice. The asterisk indicates that the salivation response to *l*-isoproterenol injected 5 min after 300 µg/kg PGE_1 was significantly less ($p < .05$) than the control response to *l*-isoproterenol injected 5 min after saline (0 dose of PGE_1).

of an agonist abolished the salivation response to *l*-norepinephrine after cocaine and to an indirectly acting amine (*d*-amphetamine), but not to a directly acting amine, *l*-isoproterenol. The temperature responses were positive and T_{MAX} values increased with elevated ambient temperatures. In this sense, reserpine may be termed not only a hypothermic, but a poikilothermic drug.

Responses to *d*-amphetamine are expected to be reduced or abolished after adequate doses of reserpine which virtually deplete the norepinephrine content of tissues. This depletion, at least in the cell bodies of the brain, is more complete at 4 hr than at 24 hr, although the nerve terminals still remain depleted at the latter time (6). Thus, it is reasonable to expect a marked effect 4 hr after reserpine. Haggendal and Lindqvist (7, 8) have shown that norepinephrine levels in brain and heart of rabbits are approximately the same 4 or 28 hr after reserpine; after 4 hr, however, the rabbits showed clear-cut signs of depression and other physiological effects, but at 28 hr, they were almost normal. It is not surprising, therefore, that responses to *d*-amphetamine were absent at 4 hr but not at 24 hr. The complete abolition of salivation responses to *d*-amphetamine 4 hr after reserpine is less surprising than the accompanying positive temperature responses, since the hyperthermic effects of *d*-amphetamine are also caused by norepinephrine release (9).

The sialogogue effect of the *l*-norepinephrine–cocaine combination was also abolished by reserpine pretreatment (Fig. 1). Cocaine, therefore, does not potentiate salivary responses to *l*-norepinephrine in the mouse pretreated with reserpine. This is difficult to understand since Furchgott *et al.* (10) reported the same degree of potentiation of norepinephrine by cocaine on aortic strips from normal and reserpine-pretreated rabbits. Perhaps cocaine blocks the rapid uptake of *l*-norepinephrine into the storage granules, or possibly extragranularly (10, 11). Anden, Magnusson and Waldeck (12) have suggested that a better correlation exists between adrenergic function and norepinephrine uptake than between adrenergic function and norepinephrine levels. Although it has been reported (13) that cocaine prevents the uptake of norepinephrine into the hearts of rats pretreated with reserpine, desmethylimipramine, another potent inhibitor in untreated animals of norepinephrine uptake, is ineffective in rats pretreated with reserpine (14). It is therefore conceivable that cocaine is ineffective in inhibiting norepinephrine uptake in mice after treatment with reserpine. Although in untreated mice, the potentiation by cocaine of salivation induced by *l*-norepinephrine was far greater than that found by Furchgott *et al.* (10) on the rabbit aortic strip, the potentiation was completely absent in the mouse pretreated with reserpine. Since the dose of *l*-norepinephrine administered in our experiments was itself insufficient to produce salivation, we have to implicate a deficiency of the cocaine effect 4 hr after reserpine. A directly acting adrenergic agonist, *l*-isoproterenol, produced salivation responses in the mouse pretreated with reserpine; this finding indicates that the adrenergic receptor mechanisms were functioning.

Whether or not *d*-amphetamine is capable

of producing salivation in mice appears to depend on the body temperature. *d*-Amphetamine caused salivation in mice, which had been pretreated with either reserpine or saline, if T_{MAX} values exceeded a critical level. Likewise, reserpine pretreatment significantly decreased but did not abolish heat-induced salivation. Since the last hour of the 4 hr reserpine pretreatment was at the elevated ambient temperature, it is possible that this factor increased norepinephrine synthesis.

The inhibition of salivation responses to *d*-amphetamine by α-MT can also be explained in terms of the reduced norepinephrine content of the tissues (15). It is surprising that the significant inhibition of salivation responses was not accompanied by significant reduction of T_{MAX} values (Fig. 1).

In a previous paper (1), we have presented evidence suggesting "that heat-induced salivation is mediated through adrenergic receptors in the salivary glands, which are inhibited by nicotinic receptor ganglionic blocking agents and beta adrenergic blocking agents. It is unlikely that heat-induced salivation is cholinergic since cholinergic salivation is unaffected by the nicotinic receptor blocking agent, chlorisondamine, which suppresses heat-induced salivation completely." The reduced salivation response to heat exposure in mice pretreated with reserpine also supports the concept that heat-induced salivation is adrenergic.

Several mechanisms may be involved in the inhibition of adrenergic salivation by PGE_1. With higher doses, the lowering of body temperature may contribute to the inhibition of salivation responses to *d*-amphetamine (Fig. 4). It should be emphasized, however, that doses of PGE_1 too small to affect body temperature inhibited salivation responses to *d*-amphetamine almost as effectively as the larger hypothermic doses. This would suggest an adrenergic receptor blocking action (2, 3) of PGE_1, except for the fact that the salivation and temperature responses to *l*-norepinephrine after cocaine were unaffected by PGE_1 (Fig. 5) and the salivation responses to *l*-isoproterenol were reduced less than 35%. The greater inhibition of responses to *d*-amphetamine indicates therefore that PGE_1 may be blocking the release of norepinephrine by the indirectly acting amine in addition to a direct blockade of the adrenergic receptors.

In contrast to reserpine, PGE_1 does not abolish the salivation response to *l*-norepinephrine plus cocaine. This may be related to the failure of PGE_1 to reduce the T_{MAX} value in response to *l*-norepinephrine after cocaine.

Summary. Pretreatment with reserpine, α-MT, or PGE_1 reduced or abolished salivation responses to *d*-amphetamine. Reserpine also abolished salivation responses to *l*-norepinephrine after cocaine, but not to the directly acting amine, *l*-isoproterenol. PGE_1 was most effective in reducing salivation responses to *d*-amphetamine, less effective against *l*-isoproterenol, and ineffective against *l*-norepinephrine after cocaine. Elevated ambient temperature prevented the effects of reserpine pretreatment on salivation responses to *d*-amphetamine. Reserpine pretreatment likewise only reduced, but failed to block, heat-induced salivation. High body temperatures favor while low body temperatures antagonize adrenergic salivation responses, especially those to *d*-amphetamine.

The authors thank Mr. Morton Raff of the Biometrics Research Branch of the National Heart and Lung Institute for the statistical analyses of the data.

1. Maling, H. M., and Koppanyi, T., Arch. Int. Pharmacodyn. Ther., in press.

2. Bergstrom, S., Carlson, L. A., and Weeks, J. R., Pharmacol. Rev. **20**, 1 (1968).

3. Clegg, P. C., Nature (London) **209**, 1137 (1966).

4. Maling, H. M., Williams, M. A., and Koppanyi, T., Arch. Int. Pharmacodyn. Ther., in press.

5. Scheffe, H., "The Analysis of Variance," 477 pp. Wiley, New York (1959).

6. Dahlstrom, A., Fuxe, K., and Hillarp, N., Acta Pharmacol. Toxicol. **22**, 277 (1965).

7. Haggendal, J., and Lindqvist, M., Acta Physiol. Scand. **57**, 431 (1963).

8. Haggendal, J., and Lindqvist, M., Acta Physiol. Scand. **60**, 351 (1964).

9. Gessa, G. L., Clay, G. A., and Brodie, B. B., Life Sci. **8**, 135 (1969).

10. Furchgott, R. F., Kirpekar, S. M., Rieker, M., and Schwab, A., J. Pharmacol. Exp. Ther. **142**, 39

(1963).

11. Carlsson, A., and Waldeck, B., J. Pharm. Pharmacol. **18**, 252 (1966).

12. Anden, N., Magnusson, T., and Waldeck, B., Life Sci. **3**, 19 (1964).

13. Lindmar, R., and Muscholl, E. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. **247**, 469 (1964).

14. Iversen, L. I., Glowinski, J., and Axelrod, J., J. Pharmacol. Exp. Ther. **150**, 173 (1965).

15. Corrodi, H., and Hanson, L. C. F., Psychopharmacologia **10**, 116 (1966).

Received Dec. 20, 1972. P.S.E.B.M., 1972, Vol. 140.