Biochemical Pharmacology, 1964, Vol. 13, pp. 1263–1265. Pergamon Press Ltd., Printed in Great Britain.

Tachyphylaxis to norepinephrine and its modification by cocaine*

(*Received* 9 *February* 1964; *accepted* 10 *April* 1964)

IT HAS been demonstrated in a previous study that acute tolerance (tachyphylaxis) to the pressor effects of epinephrine occurs when a constant amount of this amine is repetitively injected at a fixed rate of administration.[1] The mechanism or mechanisms responsible for this phenomenon are not completely and definitively known. Nevertheless, the work of several investigators has yielded indirect evidence which indicates that the cause of tachyphylaxis is the gradual and progressive saturation of the adrenergic receptors by the sympathomimetic amine molecules.[2, 3]

This hypothesis states that the speed at which the adrenergic receptors become saturated is a function of the plasma concentration of catecholamine molecules. Therefore, if this hypothesis is correct, it is to be expected that when the plasma levels of catecholamines are increased, the speed at which receptor saturation (tachyphylaxis) occurs should become proportionately accelerated.

Several factors regulate the plasma concentration of sympathomimetic amines; some exert their influence directly, like the amount of epinephrine given per injection, and others indirectly, like the mechanisms that inactivate circulating catecholamines. It has been demonstrated that if the amount of epinephrine given per injection is increased or if the mechanisms responsible for the inactivation of circulating catecholamines are pharmacologically blocked, the speed at which tachyphylaxis develops becomes significantly accelerated.[1]

Binding and storage of circulating catecholamines at the sympathetic nerve endings appears to be quantitatively a more important mechanism of inactivation for norepinephrine than for epinephrine. Thus, Whitby *et al.*,[4] in experiments performed on cats and mice, found that the sympathetic storage mechanism inactivates a larger proportion of ^{3}H-norepinephrine than of ^{3}H-epinephrine, and that O-methylation inactivates conversely a greater proportion of ^{3}H-epinephrine than of ^{3}H-norepinephrine. It is conceivable that, in the whole animal, the combined action of metabolic degradation and binding at the sympathetic storage sites might result in similar rates of inactivation for each of these two amines. That is to say, the rate of inactivation of a given amount of epinephrine per unit of time could be similar to the rate of inactivation of an equivalent amount of norepinephrine. If this is true, it can be expected that when groups of cats are repetitively injected with a given amount of norepinephrine, the speed at which the adrenergic receptors become saturated in each of the groups should be similar. On the other hand, if epinephrine were inactivated at a slower rate than norepinephrine, as suggested by Kirpekar *et al.*,[5] then it would follow that tachyphylaxis to norepinephrine would develop more slowly than the tachyphylaxis to epinephrine. Furthermore if, as mentioned above, binding at the sympathetic storage sites is comparatively a more important mechanism for the inactivation of norepinephrine than for epinephrine, the blockade of this mechanism by cocaine should accelerate norepinephrine tachyphylaxis much more than it does epinephrine tachyphylaxis.

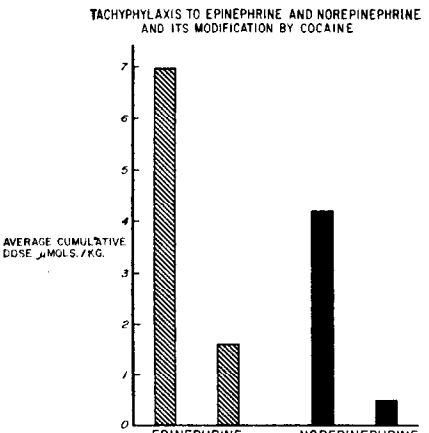
To test these possibilities, experiments were performed in 32 cats weighing between 2 and 4·5 kg. The animals were anesthetized with sodium pentobarbital (25 mg/kg, i.p.). One femoral vein and artery were catheterized, both vagus nerves sectioned, and a tracheotomy performed. The arterial blood pressure and respiration were continuously recorded by means of a system of Statham transducers and a type RP Offner polygraph.

Tachyphylaxis was induced by the rapid i.v. injection of 50 μg epinephrine HCl/kg (dose equivalent to 0·22 μmole/kg) in 22 cats, and of 50 μg norepinephrine bitartrate/kg (dose equivalent to 0·15 μmole/kg) in 10 cats. The dose volume was always 1 ml and was injected within 10 sec at intervals of 5 min in all experiments. After each injection the system was flushed with 2 ml of normal saline solution.

* This investigation was supported by Grant MH-06658 of the National Institute of Mental Health.

Cocaine HCl (5 mg/kg), dissolved in 5 ml of normal saline, was injected i.v. 15 min before the induction of tachyphylaxis in 8 of the cats in which epinephrine was tested and in 5 of the cats in which norepinephrine was tested.

The catecholamines were repetitively injected until the magnitude of the systolic blood pressure response became 33% or less of the initial response. When this point was reached, it was considered that tachyphylaxis had occurred. The number of injections necessary to reach this point measures the speed at which tachyphylaxis develops.



	EPINEPHRINE		NOREPINEPHRINE	
	CONTROLS	COCAINE	CONTROLS	COCAINE
NUMBER OF CATS	14	8	5	5
AVERAGE NUMBER OF INJECTIONS	31.64	7.25	27.8	3.2
AVERAGE CUMULATIVE DOSE μMOLS./KG.	6.96	1.60	4.17	0.48

FIG. 1. Because the doses of epinephrine and norepinphrine administered per injection were not equivalent, the average cumulative dose (i.e., the amount of epinephrine or norepinephrine necessary to induce tachyphylaxis) was converted to micromoles per kilogram and used as a basis for comparison. In addition, the number of cats utilized in each of the experimental situations and the number of times that a constant amount of each of the amines was injected (50 μg/kg) are illustrated.

The results are summarized in Fig. 1. It is observed that epinephrine control cats required an average cumulative dose of 6·96 μmoles/kg and norepinephrine control cats an average cumulative dose of 4·17 μmoles/kg to reach tachyphylaxis, respectively. The difference between the groups was analyzed statistically by parametric and nonparametric techniques, and it was found not to be significant ($P > 0{\cdot}10$).

This finding indicates that the speed at which tachyphylaxis developed was essentially similar for epinephrine and norepinephrine and argues strongly in favor of the hypothesis that the rate at which these two amines are inactivated in the cat (sum total of metabolic degradation and binding at the sympathetic storage sites) is similar. Thus, if receptor saturation is accepted as the causal mechanism of catecholamine tachyphylaxis, it can be inferred that the rate at which the adrenergic receptors became saturated was similar for both epinephrine and norepinphrine. Moreover, if it is accepted that the rate at which adrenergic receptors saturate is a function of the plasma concentration of catecholamines, and that this concentration is itself dependent on the rate of catecholamine inactivation, then it follows that in our experiments epinephrine and norepinephrine must have been inactivated at similar rates.

In the second part of these experiments the action of cocaine upon the speed at which tachyphylaxis to epinephrine and norepinephrine develops was investigated. Figure 1 indicates that cats treated with cocaine required an average cumulative dose of 1·60 μmoles epinephrine/kg and 0·48 μmoles norepinephrine/kg to become tachyphylactic, respectively. These results indicate that epinephrine-cocaine treated cats required 23% of the average cumulative dose of the epinephrine controls,

and norepinephrine–cocaine treated cats required 11·5% of the average cumulative dose of the norepinephrine controls, respectively, to become tachyphylactic.

Statistical analysis of these data by parametric and nonparametric techniques shows that the difference between the epinephrine controls and the epinephrine–cocaine treated cats ($P < 0.001$), between the norepinephrine controls and the norepinephrine–cocaine treated cats ($P < 0.01$), and between the epinephrine and the norepinephrine–cocaine treated cats ($P < 0.05$), were all significant.

These findings indicate that cocaine markedly accelerated the rate of development of tachyphylaxis to epinephrine and norepinephrine, and that the speed at which tachyphylaxis to norepinephrine developed was significantly more accelerated than that to epinephrine.

It is generally accepted that cocaine blocks the uptake and storage of catecholamines by the sympathetic nerve endings.[5–8] The immediate consequence of this effect is a reduction in the capacity to inactivate circulating sympathomimetic amines. Thus, after cocaine, the injection of a given amount of epinephrine or norepinephrine cannot be effectively disposed of by metabolic degradation alone, and the plasma concentration of these amines remains higher for a longer period of time than occurs in control animals. This has been demonstrated by Hertting *et al.*,[9] Muscholl,[10] and Whitby *et al.*,[11] who have shown that cocainization resulted in a higher plasma concentration and a lower tissue concentration of tritium-labeled catecholamines than occurred in control animals. Therefore, it seems valid to assume that when catecholamines are repetitively infused the adrenergic receptors of animals treated with cocaine are exposed to larger concentrations of active catecholamine molecules and become more rapidly saturated than do the receptors of control animals.

The finding that cocaine accelerated the rate of development of tachyphylaxis more significantly to norepinephrine than to epinephrine suggests that, in the cat, binding and storage at the sympathetic nerve endings is quantitatively a more important mechanism for inactivating exogenous norepinephrine than exogenous epinephrine.

The acceleration of the rate of development of epinephrine tachyphylaxis produced by the cocaine-blockade of the sympathetic storage sites is greater than the acceleration obtained by the pharmacological blockade of either O-methylation or oxidative deamination (unpublished observations). These findings suggest that the capacity of the storage mechanism in sympathetic nerve endings for inactivating large quantities of catecholamines is greater than that of metabolic degradation.

Until determinations of the tissue and plasma concentration of catecholamines and their metabolite. at various stages of the development of tachyphylaxis are actually made, the hypotheses proposed must remain speculative.

Acknowledgement—The technical assistance of Mrs. Jeanne White, Mr. Richard Doughten, and Mr. Rudolph Barker is gratefully acknowledged.

Department of Psychiatry,
University of North Carolina,
School of Medicine,
Chapel Hill, N.C., U.S.A.

MARIO PEREZ-REYES

REFERENCES

1. M. PEREZ-REYES and M. LIPTON, *Proc. Soc. exp. Biol. (N. Y.)* **112**, 181 (1963).
2. M. H. SEEVERS and L. A. WOODS, *Amer. J. Med.* **4**, 546 (1953).
3. C. V. WINDER, M. M. ANDERSON and H. C. PARKE, *J. Pharmacol.* **93**, 63 (1948).
4. L. G. WHITBY, G. HERTTING and J. AXELROD, *Nature, Lond.* **187**, 604 (1960).
5. S. M. KIRPEKAR, P. CERVONI and R. F. FURCHGOTT, *J. Pharmacol.* **93**, 63 (1948).
6. W. H. MACMILLARN, *Brit. J. Pharmacol.* **14**, 385 (1959).
7. J. H. BURN and M. J. RAND, *Advanc. Pharmacol.* 2 (1962).
8. L. T. POTTER and J. AXELROD, *J. Pharmacol.* **140**, 199 (1963).
9. G. HERTTING, J. AXELROD and L. G. WHITBY, *J. Pharmacol.* **134**, 146 (1961).
10. E. MUSCHOLL, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **240**, 8 (1960).
11. L. G. WHITBY, J. AXELROD and H. WEIL-MALHERBE, *J. Pharmacol.* **132**, 193 (1961).