Tachyphylaxis III

Ephedrine

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Tachyphylaxis to ephedrine was studied in anesthetized cats by the following parameters: pressor responses (height and duration), heart rate, nictitating membrane contractions, and tone. *Levo* or *dl*-norepinephrine infusions, in contrast to epinephrine or phenylephrine infusions, given after the initial development of tachyphylaxis, produced a partial return of responses to ephedrine. Ephedrine tachyphylaxis and its specific reversal by norepinephrine are discussed.

OUR STUDIES on the reversal of neuro-sympathomimetic amine tachyphylaxis (1, 2) were continued with *l*-ephedrine sulfate, which is a well known tachyphylactogenic agent (3, 4). Its action has been postulated to be of a mixed nature (5–7), *i.e.*, it appears to act directly on smooth muscle as well as indirectly by release of norepinephrine from endogenous stores. Thus, it was of particular interest to ascertain whether the replacement of the partially depleted neurohumor, *l*-norepinephrine, would alter the development of tachyphylaxis to this sympathomimetic amine of dual action.

METHODS

l-Ephedrine sulfate was injected intravenously once every hour at a dose of 1.5 mg./Kg. or 0.75 mg./Kg. *l*-Norepinephrine bitartrate monohydrate (*l*-NOR) or *dl*-norepinephrine hydrochloride (*dl*-NOR) were dissolved in physiological salt solution to a concentration of 60 mcg./ml. and administered intravenously by continuous infusion at the rate of 2.3 mcg./minute, regardless of the weight of the animal. The infusions started 15 minutes after the second, third, or fourth ephedrine injection when tachyphylaxis was developed. In view of the differences in molecular weights between the two norepinephrine salts and of the well known lesser potency of *d*-norepinephrine, this infusion rate was arbitrarily chosen.

To ascertain the specificity of norepinephrine, phenylephrine hydrochloride (diluted to 300 mcg./ml. with physiological salt solution) was infused starting 15 minutes after the second hourly ephedrine injection at a rate of 11.6 mcg./minute, as was *l*-epinephrine hydrochloride at a rate of 4.6 mcg./minute. Dichloroisoproterenol hydrochloride (DCI), the β-receptor blocking agent (9), was administered intravenously at a dose of 3 mg./Kg. 15 minutes before the first hourly injection of ephedrine to determine the influence of β-receptors (8).

Male cats, weighing 3–4 Kg., were anesthetized with α-chloralose (80.0 mg./Kg. intraperitoneally) and pretreated with atropine sulfate (2.0 mg./Kg. intravenously). Polygalacturonic acid glycoside (PAG) was employed as an anticoagulant. The parameters measured and reported in this paper were (*a*) mean arterial blood pressure, (*b*) heart rate, (*c*) tonus, and (*d*) contractions of the nictitating membrane. Blood pressure from the left carotid artery was recorded by a Sanborn transducer (No. 267-B) on a four channel Sanborn polygraph. The changes in blood pressure responses are reported in mm. Hg and their duration in minutes. The contractions and tone of the right nictitating membrane in animals were recorded with a Grass transducer (No. FTO3) with the crystalline lens removed. The weight on the nictitating membrane was 3.2 Gm. The attenuator (Carrier preamplifier, Sanborn No. 150-1100AS) was set at $\times 5$; the responses of the nictitating membrane are reported in millimeters and read directly from the paper. Changes in heart rate were counted from the blood pressure recording with the paper speed set at 5 mm./second. The right femoral vein was cannulated for single injections and the left femoral vein for continuous drug infusions. Tracheotomy was routinely performed in all animals, although it was not necessary to use artificial respiration in any of the animals.

The first ephedrine injection was given 15 minutes after completion of surgery and pretreatment with atropine and PAG.

The data were evaluated statistically and the control animals were compared with the infused cats by the "t" test for paired experiments (10).

MATERIALS

The following drugs were used in these experiments: *l*-ephedrine sulfate,[1] *l*-norpinephrine bitartrate monohydrate,[2] *dl*-norepinephrine hydrochloride,[3] phenylephrine hydrochloride,[4] *l*-epinephrine hydrochloride,[5] dichloroisoproterenol hydrochloride,[6] α-chloralose,[7] atropine sulfate,[8] and polygalacturonic acid glycoside.[9] All doses are expressed as the weight of the respective salts.

RESULTS

Ephedrine Sulfate, 0.75 mg./Kg.

For the following reported experiments, only

Received November 21, 1962, from the Department of Pharmacology, Medical Center, Georgetown University, Washington, D. C.

Accepted for publication February 6, 1963.

Supported in part by Grant B-1448 from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service, by General Services grant of Georgetown University, and by Schering Corp. grant.

[1] New York Quinine Co., New York, N. Y.
[2] Biochemicals Corp., Cleveland, Ohio.
[3] Sigma Chemical Corp., St. Louis, Mo.
[4] Winthrop Laboratories, New York, N. Y.
[5] Marketed as Adrenalin by Parke, Davis and Co., Detroit, Mich.
[6] Eli Lilly and Co., Indianapolis, Ind.
[7] Kuhlmann Products, Paris, France.
[8] New York Quinine Co., New York, N. Y.
[9] Marketed as Mepesulfate by Hoffmann-LaRoche, Nutley, N. J.

TABLE I.—DIFFERENCES IN RESPONSES TO HOURLY INTRAVENOUS INJECTIONS OF 0.75 MG./KG. OF EPHEDRINE WITH [a] AND WITHOUT [b] CONTINUOUS INTRAVENOUS INFUSION OF *l*-NOREPINEPHRINE (*l*-NOR)

Differences in responses	Treatment	$\bar{d} \pm s\bar{d}$, 3rd to 4th	*P*, 3,4	$\bar{d} \pm s\bar{d}$, 3rd to 5th	*P*, 3,5	$\bar{d} \pm s\bar{d}$, 3rd to 6th	*P*, 3,6	$\bar{d} \pm s\bar{d}$, 3rd to 7th	*P*, 3,7
Blood pressure, mm. Hg	Without *l*-NOR	-6 ± 2.2	<0.05[c]	-6 ± 3	<0.2	-12 ± 2.9	<0.02[c]	-16 ± 0.7	<0.001[c]
	With *l*-NOR	-3.7 ± 4.6	<0.5	-9.3 ± 5.5	<0.2	-13.1 ± 6.4	<0.2	-8.1 ± 6.2	<0.3
Heart rate, beats/min.	Without *l*-NOR	-9.6 ± 2.4	<0.02[c]	-12.4 ± 3.5	<0.05[c]	-14.4 ± 2.4	<0.01[c]	-14.4 ± 2.4	<0.001[c]
	With *l*-NOR	$+3 \pm 3.4$	<0.5	$+3 \pm 6$	<0.7	-9 ± 3	<0.1	-9 ± 3	<0.1

[a] Number of cats, 4; weight, 3.0 to 3.5 Kg. [b] Number of cats, 5; weight 3.0 to 3.5 Kg. [c] Indicates statistical significance.

those cats were chosen which showed clear-cut tachyphylaxis after the third hourly injection. This group of animals was comprised of 13 cats, five of which were used as controls and eight of which were infused with norepinephrine.

A dose of 0.75 mg./Kg. of ephedrine caused invariably reduced pressor responses to the second hourly injection when compared with the first, but in many animals the responses thereafter showed very little (if any) additional decrease. In some cats this dose of ephedrine caused continued tachyphylaxis on blood pressure as could be judged from the substantial diminution of the pressor response to the injections thereafter. The duration of the responses also decreased with the repeated injections of ephedrine, from an average of 6 minutes to an average of 2 minutes.

Infusion With *l*-Norepinephrine (*l*-NOR).—With the infusion rate of *l*-NOR employed, the initial mean arterial blood pressure was little changed but decreased slightly toward the end of the experiments.

The duration of the blood pressure responses to ephedrine was not altered by the infusion of *l*-NOR when compared with the controls.

In Table I, the differences in the blood pressure responses and changes in heart rate from the fourth through seventh hourly injections of ephedrine in the absence and presence of *l*-NOR are compared with the third hourly responses. Five cats received only hourly ephedrine injections (controls), whereas four cats were continuously infused intravenously with *l*-NOR starting after the third hourly injection of ephedrine.

As can be seen from Table I, the decrease in the blood pressure responses to ephedrine in the control animals became progressively more significant with time, except for the fifth hour. The differences between the responses in the *l*-NOR-treated animals were not significant throughout this period of time, showing that *l*-norepinephrine prevented the further development of ephedrine tachyphylaxis. Also the heart-rate changes in the control animals were significantly reduced and *l*-NOR infusion counteracted this reduction.

The nictitating membrane contractions induced by ephedrine did not show such a clear-cut effect of *l*-NOR. In all of the control animals the contractions were progressively and significantly reduced. When compared with the third hourly response, *l*-NOR showed its reversing effect only at the fourth and sixth hours. In the control animals the nictitating membrane tone increased significantly in the fourth and fifth hours and thereafter remained essentially the same. In the *l*-NOR treated animals, the nictitating membrane tone plateaued after the fourth hourly injection of ephedrine.

Infusion with *dl*-Norepinephrine (*dl*-NOR).—The infusion of *dl*-NOR in doses already referred to did not alter the initial mean arterial blood pressure significantly. The duration of the blood pressure responses to ephedrine was essentially unaltered by the infusion of *dl*-NOR.

The effect of infusion of *dl*-NOR on the changes in blood pressure response, the changes in heart rate, and the nictitating membrane tone to ephedrine was identical with that described previously for *l*-NOR. The effect of *dl*-NOR on the nictitating membrane contractions to ephedrine is summarized in Table II.

As can be clearly seen, the nictitating membrane contractions to ephedrine show significant reductions upon the progressive hourly administration of ephedrine in the control cats and *dl*-NOR prevents this reduction. This shows that *dl*-NOR, in contrast to *l*-NOR, is capable of counteracting ephedrine tachyphylaxis on this organ.

Ephedrine Sulfate, 1.5 mg./Kg.

A dose of 1.5 mg./Kg. of ephedrine caused significant reductions of the mean arterial blood pressure and heart-rate responses upon repeated hourly injections in all control animals.

TABLE II.—DIFFERENCES OF NICTITATING MEMBRANE CONTRACTIONS (MM.) TO 0.75 MG./KG. OF EPHEDRINE INJECTED INTRAVENOUSLY ONCE EVERY HOUR

Treatment	$\bar{d} \pm s\bar{d}$, 3rd to 4th	*P*, 3,4	$\bar{d} \pm s\bar{d}$, 3rd to 5th	*P*, 3,5	$\bar{d} \pm s\bar{d}$, 3rd to 6th	*P*, 3,6	$\bar{d} \pm s\bar{d}$, 3rd to 7th	*P*, 3,7
Without *dl*-NOR[a]	-2.4 ± 0.48	<0.01[c]	-3.7 ± 0.7	<0.01[c]	-4.6 ± 1.3	<0.05[c]	-5.1 ± 1.3	<0.02[c]
With *dl*-NOR[b,d]	$+1.5 \pm 1.2$	<0.3	$+0.9 \pm 1.5$	<0.6	$+0.5 \pm 1.2$	<0.7	-0.8 ± 0.9	<0.5

[a] Number of cats, 5; weight, 3.0 to 4.5 Kg. [b] Number of cats, 4; weight, 3.0 to 4.0 Kg. [c] Indicates statistical significance. [d] *dl*-Norepinephrine (*dl*-NOR) was continuously infused intravenously starting 15 minutes after the third hourly and ending 30 minutes after the seventh hourly ephedrine injection at the rate of 2.3 mcg./min.

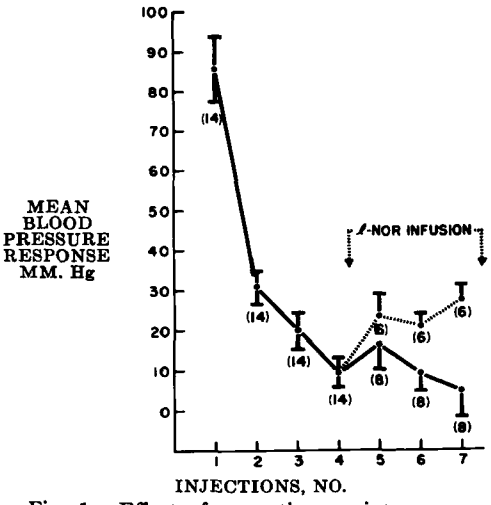

Fig. 1.—Effect of a continuous intravenous infusion of *l*-norepinephrine (*l*-NOR, 2.3 mcg./minute) upon the mean arterial blood pressure responses to ephedrine (1.5 mg./Kg.) injected intravenously once every hour. The *l*-norepinephrine infusion was started 15 minutes after the fourth and ended 30 minutes following the seventh hourly ephedrine injection in six of 14 cats. The remaining eight animals were given only the hourly injections of ephedrine (controls). The pressor responses to ephedrine of the control and *l*-NOR-infused groups of cats were pooled for the first four hourly injections.

With this dose of ephedrine the initial mean arterial blood pressure increased slightly during the course of the experiments in all control cats (average, +28 mm. Hg).

The duration of the blood pressure responses to this dose of ephedrine showed a greater reduction than that observed, following the repeated hourly administration of the lower dose (0.75 mg./Kg.), *i.e.*, from an average of 8 minutes to an average of less than 1 minute.

Infusion with *l*-Norepinephrine (*l*-NOR).—The rate of infusion of *l*-norepinephrine, 2.3 mcg./minute, was determined in pilot studies and was used in these experiments. When infusion rates of *l*-NOR *smaller* than 2.3 mcg./minute were employed in six cats, complete tachyphylaxis to the pressor effects of ephedrine developed. When a *higher* infusion rate of *l*-NOR was used in six cats, the initial pressor responses to ephedrine following *l*-norepinephrine infusions were considerably augmented. However, *l*-norepinephrine at these *higher* infusion rates did not prevent the terminal development of tachyphylaxis. *l*-NOR infusion at the rate of 2.3 mcg./minute increased the initial mean arterial blood pressure by an average of +25 mm. Hg, which remained essentially constant throughout the experiments.

The duration of the blood pressure responses to ephedrine in the presence of *l*-NOR was less reduced than in the control animals (from an average of 8 minutes to an average of 2 minutes).

Figure 1 summarizes the pooled mean arterial blood pressure changes for all 14 animals regarding the first four hourly injections of ephedrine. Eight of these 14 animals served as the ephedrine controls. In the remaining six animals *l*-NOR infusion (2.3 mcg./minute) was started 15 minutes after the fourth injection and continued to the end of the experiment. It can be clearly seen that the infusion *l*-NOR *at this rate* will reverse tachyphylaxis to the level of the second hourly injection of ephedrine but not fully. Thus, the reversal of tachyphylaxis in this experiment is partial but not complete. The "protective" effect of *l*-NOR on the pressor responses to ephedrine (fifth to the seventh hour) is statistically significant (P = <0.02 to <0.01). In summary, we may say that an infusion rate of *l*-NOR of 2.3 mcg./minute was effective in reversing the development of tachyphylaxis of the ephedrine pressor responses, but that infusion rates *higher* or *lower* than 2.3 mcg./minute were not effective.

The data for the changes of heart rate in these same animals are summarized in Fig. 2. The changes in heart rate to ephedrine become progressively less reduced to repeated injections of the amine after the second hour.

Between the first and second injections of ephedrine the heart rate is elevated and remains rapid throughout the experiment.

The continuous infusion of *l*-NOR partially reverses the ever decreasing heart-rate response to ephedrine, although this reversal is statistically significant only at the seventh injection of ephedrine (P = <0.02).

A summary of the nictitating membrane tone and contractions of these same animals is given in Table III.

The nictitating membrane responses to ephedrine of all 14 animals were pooled for the first 4 hours, and the responses of the eight control animals and the six *l*-NOR-infused animals are shown separately for the fifth through the seventh hour. As can be seen, the sums of the nictitating membrane contraction and tone are nearly constant throughout the experimental period, both in the presence and absence of *l*-NOR.

From the fifth to the seventh hour the eight control cats show a progressive decrease of the nictitat-

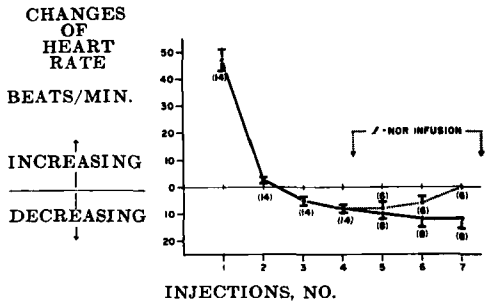

Fig. 2.—Effect of a continuous intravenous infusion of *l*-norepinephrine (*l*-NOR, 2.3 mcg./minute) upon the ephedrine-induced (1.5 mg./Kg. intravenously) mean changes in heart rate. The ephedrine was injected once every hour. The *l*-norepinephrine infusion was started 15 minutes after the fourth and ended 30 minutes following the seventh hourly ephedrine injection in six of 14 cats. The remaining eight animals were given only the hourly injections of ephedrine (controls). The ephedrine-induced changes in heart rate observed in the control and *l*-NOR-infused groups of cats were pooled for the first four hourly injections.

TABLE III.—NICTITATING MEMBRANE RESPONSES (MM.) TO 1.5 MG./KG. OF EPHEDRINE INJECTED INTRAVENOUSLY ONCE EVERY HOUR[a]

Injection, No. →	1	2	3	4	5	6	7
Without *l*-NOR (No. cats, 8)							
1. Contraction	14.8 ± 0.8	7.0 ± 0.6	4.8 ± 0.4	3.8 ± 0.4	4.8 ± 0.4	3.8 ± 0.6	3.2 ± 0.8
2. Tone	7.8 ± 0.06	17.6 ± 0.6	19.4 ± 0.8	19.0 ± 0.6	17.6 ± 0.8	18.0 ± 1.2	18.6 ± 1.4
3. Sum of 1 + 2	22.4	24.6	24.2	22.8	22.4	21.8	21.8
With *l*-NOR[b] (No. cats, 6)							
4. Contraction	...	...	...	...	2.6 ± 0.4	2.6 ± 0.4	3.0 ± 0.4
5. Tone	...	...	...	...	20.2 ± 0.6	20.4 ± 1.2	19.6 ± 1.2
6. Sum of 4 + 5	...	...	...	...	22.8	23.0	22.6

[a] The responses of all 14 cats were pooled for the first four hourly ephedrine injections. [b] *l*-Norepinephrine (*l*-NOR) was continuously infused intravenously starting 15 minutes after the fourth and ending 30 minutes after the seventh hourly ephedrine injection at the rate of 2.3 mcg./min.

ing membrane contractions and a simultaneous increase in tone. The nictitating membrane tone of the *l*-NOR-treated cats is higher than that of the control animals, and the contractions are lower. Nevertheless, *l*-NOR might exert a slight "protective" effect since a further decrease in contractions from the fifth to the seventh hour is absent. The "protective" effect of *l*-NOR on these nictitating membrane contractions to ephedrine cannot be demonstrated by statistical methods.

We questioned whether repeated hourly injections of 1.5 mg./Kg. of ephedrine would depress the responses to single doses of *l*-NOR.

Ephedrine tachyphylaxis was permitted to develop through the fourth injection. Single repeated (three times) injections of a geometric series of *l*-NOR (0.25 to 1.0 mcg./Kg.) were administered intravenously before the first and after the fourth ephedrine injections. The pressor responses to the single injections of *l*-NOR were biphasic and ranged *initially* from +77 to −3 mm. Hg to the low dose, to +103 to −19 mm. Hg to the high dose of *l*-NOR. After the fourth ephedrine injection, the responses to the low dsse of *l*-NOR were +73 to −12 mm. Hg and to the high dose +86 to −9 mm. Hg. Thus, in the presence of ephedrine tachyphylaxis only insignificant reductions of the pressor responses to the single injections of *l*-NOR could be observed.

Infusion with *dl*-Norepinephrine (*dl*-NOR).—In addition to *l*-NOR, *dl*-NOR was also employed to study its effect on the development of ephedrine tachyphylaxis. The infusion of *dl*-NOR essentially failed to alter the initial mean arterial blood pressure at the rates administered.

dl-NOR was infused (2.3 mcg./minute) starting 15 minutes after the fourth hourly injection of ephedrine, and this catecholamine infusion was continued to the end of each experiment. It is obvious that *dl*-NOR is less potent than *l*-NOR; nevertheless, the results observed on the pressor responses to ephedrine are similar, though the heart rate responses appear to be less marked. Furthermore, *dl*-NOR caused only an insignificant increase in the duration of the blood pressure responses to ephedrine when compared with control animals. (Average first response, 10 minutes; average seventh response, 1.1 minutes.)

It may be observed in Table IV that in cats infused with *dl*-NOR the ephedrine-induced contractions of the nictitating membrane are significantly higher than those of the control animals. The increase in nictitating membrane tone is considerably less pronounced in the animals infused with *dl*-NOR compared with that observed in the cats infused with *l*-NOR.

Infusion with Other Sympathomimetic Amines.—To ascertain the specificity of norepinephrine in partially counteracting ephedrine tachyphylaxis, five cats were infused with *l*-epinephrine and three cats with phenylephrine (see *Materials* and *Methods*).

TABLE IV.—DIFFERENCES IN RESPONSES TO HOURLY INTRAVENOUS INJECTIONS OF 1.5 MG./KG. OF EPHEDRINE WITH[a] AND WITHOUT[b] CONTINUOUS INTRAVENOUS INFUSION OF *dl*-NOREPINEPHRINE (*dl*-NOR) STARTING 15 MINUTES AFTER THE FOURTH HOURLY EPHEDRINE INJECTION

Differences in responses	Treatment	$\bar{d} \pm \overline{sd}$, 4th to 5th	*P*, 4,5	$\bar{d} \pm \overline{sd}$, 4th to 6th	*P*, 4,6	$\bar{d} \pm \overline{sd}$, 4th to 7th	*P*, 4,7
Blood pressure, mm. Hg	Without *dl*-NOR	− 3.8 ± 3.0	<0.3	− 9.2 ± 4.9	<0.2	−15.0 ± 3.0	<0.01[c]
	With *dl*-NOR	+18.8 ± 5.6	<0.05[c]	+18.3 ± 3.8	<0.01[c]	+23.6 ± 4.8	<0.01[c]
Heart rate, beats/min.	Without *dl*-NOR	− 2.0 ± 2.0	<0.4	− 4.0 ± 1.8	<0.1	− 4.8 ± 2.9	<0.2
	With *dl*-NOR	− 2.0 ± 2.0	<0.4	− 2.0 ± 2.0	<0.4	− 2.4 ± 2.4	<0.4
Nictitating membrane contraction, mm.	Without *dl*-NOR	− 0.3 ± 0.2	<0.2	− 1.3 ± 0.4	<0.02[c]	− 2.2 ± 0.4	<0.01[c]
	With *dl*-NOR	− 0.2 ± 0.2	<0.4	− 0.6 ± 0.3	<0.1	−0.7 ± 0.4	<0.2
Nictitating membrane tone, mm.	Without *dl*-NOR	−0.03 ± 0.8	<1.0	+ 0.2 ± 0.5	<0.8	+ 0.8 ± 0.5	<0.2
	With *dl*-NOR	+ 0.1 ± 0.3	<0.7	+ 0.2 ± 0.5	<0.8	± 0.01 ± 0.7	<1.0

[a] Number of cats, 6; weight 3.0 to 4.5 Kg.; infusion rate of *dl*-NOR 2.3 mcg./min. [b] Number of cats, 8; weight 3.0 to 4.5 Kg. [c] Indicates statistical significance.

None of the four parameters measured was influenced by the infusion of these two sympathomimetic amines when compared with the controls.

Effect of Dichloroisoproterenol (DCI) upon Development of Ephedrine Tachyphylaxis

Since DCI was shown to antagonize the effects of ephedrine on the heart (11), it was of interest to test the influence of this β-receptor blocking agent on ephedrine tachyphylaxis and its reversal by norepinephrine. These experiments were carried out for the duration of six hourly ephedrine (1.5 mg./Kg.) injections, and DCI (3 mg./Kg.) was administered intravenously 15 minutes before the first ephedrine injection. Four cats were tested with DCI and ephedrine alone. In contrast to the cats not pretreated with DCI, which showed a slight increase in the initial mean arterial blood pressure throughout the experiments, these DCI-treated cats showed a slight decrease (average, −29 mm. Hg). No change in heart-rate response to ephedrine was observed throughout the experimental period, although the duration of action of DCI at this dose was reported to be considerably shorter than 6 hours (11). In two cats the initial heart rate remained unchanged; however, in the other two animals the initial heart rate increased somewhat after the third hour without further increase following the fourth, fifth, and sixth ephedrine injections. Nevertheless, tachyphylaxis development to ephedrine-induced blood pressure responses, nictitating membrane contractions, and the increase in nictitating membrane tone paralleled the effects of ephedrine in animals without DCI administration. However, the duration of the blood pressure responses to ephedrine was considerably prolonged (average of 40 minutes to the first injection and an average of 25 minutes to the sixth ephedrine injection). It should be emphasized that the considerable variations of the pressor effects to the first injection of ephedrine were largely eliminated in cats pretreated with DCI. This also resulted in lower pressor effects upon the first ephedrine injection.

Three other cats received DCI (3 mg./Kg.) 15 minutes before the first injection of ephedrine and were infused with *l*-NOR starting 15 minutes after the second ephedrine injection. This catecholamine was infused continuously to the end of the experiment. The initial mean arterial blood pressure in these animals was not significantly altered, and the duration of the pressor responses to ephedrine were essentially the same as those observed in the control animals. Under these experimental conditions *l*-NOR did not reverse the ephedrine-induced tachyphylaxis on the blood pressure. In these three cats the heart rate did not increase to ephedrine administration; therefore, even during the infusion of *l*-NOR, ephedrine failed to accelerate the heart. However, *l*-NOR itself produced a slight increase in the heart rate throughout the experiment.

The only parameter measured which showed statistically significant reversal of ephedrine tachyphylaxis by *l*-NOR infusion in the DCI-pretreated animals were the contractions of the nictitating membrane.

DISCUSSION

The above experiments demonstrating the tachyphylaxis of sympathomimetic amines were designed in accordance with the original method of Tainter (12).

For the present studies we arrived at a dose of ephedrine (1.5 mg./Kg.) to which tachyphylaxis of all parameters measured was observed in all experimental animals. We also employed a borderline tachyphylactic dose (0.75 mg./Kg.).

In ephedrine (1.5 or 2.5 mg./Kg.) tachyphylaxis (1), the main differences in the blood pressure responses to this drug occur between the first and second hourly injections and those to the heart rate between the first and third hourly administrations (first phase of tachyphylaxis). The reductions in these responses thereafter become progressively less pronounced (second phase of tachyphylaxis). The separation of the two phases of ephedrine tachyphylaxis becomes considerably less distinct with the 0.75 mg./Kg. dosage. There is little or no disagreement that norepinephrine release is involved in the indirect action of the sympathomimetic amines such as ephedrine. However, the direct action of these amines may be attributed to a reversible combination with α-adrenergic receptors (action abolished or greatly diminished by α-adrenergic blocking agents) and/or to a similar reversible combination with other unknown receptors in the smooth and cardiac muscle. Blockade of the cardiac β-receptors by DCI reduces the first blood pressure response to ephedrine and thereby makes the first phase of tachyphylaxis less pronounced without essentially altering the prolonged second phase.

With the infusion of *l*-NOR or *dl*-NOR we were able to overcome the second phase of tachyphylaxis. DCI, in the presence of which the first phase of tachyphylaxis is greatly reduced, prevented this reversing effect of norepinephrine on blood pressure. Thus, we must attribute a crucial role to the heart in this return of responses; however, the heart alone cannot fully account for the partial reversal by norepinephrine of the ephedrine tachyphylaxis because the contractions of the nictitating membrane were also partially re-established. Since by the infusion of norepinephrine we were unable to reverse the first phase of tachyphylaxis to ephedrine at 1.5 mg./Kg., and could not reverse either phase of tachyphylaxis to 2.5 mg./Kg., as previously reported (2), we must assume a partly irreversible direct, possibly toxic, action of ephedrine on the heart and smooth muscle, which becomes apparent immediately upon the first injection of the amine. It was observed in our laboratory that ephedrine in high concentration increased the permeability of the vessels of the rat-*meso*-appendix (13). Since ephedrine is a drug of prolonged duration of action, its toxicity or irreversible effect might also be due to such an alteration of vascular, *i.e.*, smooth muscle permeability. Another possibility is a depression by ephedrine of the secretion of adrenocorticoids which, according to Ramey and Goldstein (14), maintain the integrity and responsiveness of tissues in the process of reacting to norepinephrine.

The effects of the drugs on the smooth muscle of the nictitating membrane are difficult to evaluate unequivocally because the tone of this organ never returns to control levels after the first injection of ephedrine. Nevertheless, the contractions of the

nictitating membrane were partially re-established in the absence and presence of DCI. These results should make it obvious that we cannot expect all parameters made tachyphylactic by ephedrine to be simultaneously reversed (partially or completely) by an infusion of norepinephrine.

The observations made on blood pressure responses to single norepinephrine injections preceding and following ephedrine tachyphylaxis development exclude adrenergic contractile receptor saturation as an explanation for ephedrine tachyphylaxis. This is further corroborated by the fact that norepinephrine can be infused in amounts too large to prevent reversal which represents true "receptor saturation." This is not in complete agreement with the suggestions of Winder (15) and of Horita, West, and Dille (16) that "receptor saturation" is the true explanation for tachyphylaxis to sympathomimetic amines.

In view of the above considerations, full reversal of both phases of ephedrine tachyphylaxis with norepinephrine could not be expected. Nevertheless, our original postulate (that the loss of norepinephrine from critical sites as an important etiological factor in the development of sympathomimetic amine tachyphylaxis) was fully substantiated.

Norepinephrine shows a unique specificity since epinephrine cannot be substituted in its place as a reversing agent. The precursors of norepinephrine may be effective in reversing ephedrine tachyphylaxis if the synthesis of norepinephrine is not influenced by the presence of large concentrations of ephedrine. To evaluate fully the nature of ephedrine tachyphylaxis on smooth muscle, the nictitating membrane might constitute a very reliable test organ *in situ* if studied under more favorable conditions, such as by close-arterial injections. We agree with the findings of Trendelenburg, *et al.* (17), that ephedrine is neither a purely directly acting nor purely indirectly acting sympathomimetic amine, and this fact may be responsible for only the partial reversal of ephedrine tachyphylaxis by norepinephrine infusions.

In view of the dual nature of ephedrine, which is further substantiated by our observations, it is realized that this amine is not the best drug to demonstrate fully the phenomenon of tachyphylaxis reversal. We are investigating, therefore, tachyphylaxis reversal by norepinephrine of sympathomimetic amines reported to have a greater indirect effect than ephedrine (5–7, 17), *e.g.*, tyramine and amphetamine. These results have been reported in part and will be published in the near future.

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Studies of Preservatives of Poliomyelitis (Salk) Vaccine I

Benzethonium Chloride

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Several antimicrobial materials may be present in killed Salk poliomyelitis vaccine. They are (*a*) antibiotics which are added to the tissue culture to inhibit growth of bacterial contaminants without inhibiting growth of the poliomyelitis virus, (*b*) formaldehyde added to kill the virus, and (*c*) preservatives such as benzethonium chloride added to the finished vaccine to prevent the growth of bacterial and fungal contaminants. The stable antibiotics contribute considerable antibacterial activity; the formaldehyde, if not neutralized by bisulfite, is an effective, stable antibacterial agent with some antifungal activity. Benzethonium chloride adds little, if any, antibacterial activity to that obtained either by antibiotics or formaldehyde but does furnish some antifungal activity.

PRESERVATIVES for killed poliomyelitis (Salk) vaccine must be chosen with special care because the antigenic potency of the vaccine is easily destroyed. Benzethonium chloride (BEC) has been used as a preservative for poliomyelitis vaccine and has been found to cause no destruction of the virus antigen when used in concentrations of about 25 p.p.m. (1). This concentration

Received January 15, 1963, from Connaught Medical Research Laboratories, University of Toronto, Toronto, Ontario, Canada.
Accepted for publication February 7, 1963.
The authors gratefully acknowledge the technical assistance of Adolph von Seefried.