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Effects of a New Congener of Dibenamine on the Actions of Sympathomimetic Amines.*

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(Introduced by H. B. van Dyke.)

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Since the announcement by Nickerson and Goodman[1,2] of the adrenolytic and sympatholytic potency of some compounds of the nitrogen mustard series, several groups of workers have published reports of various aspects of the autonomic pharmacology of such compounds. A logical extension of the discovery of the adrenolytic properties, *viz.*, an examination of the capacity of such compounds to modify the sympathomimetic effects of substances other than epinephrine itself, was undertaken and is the subject of this report. The experiments were performed with N-benzyl-N-β-phenylisopropyl-β-chloroethylamine HCl (drug 194),† a substance closely related to Dibenamine. Wells and Rall[3] recently published an abstract of work similar in purpose.

Methods All the experiments were performed with cats (1-5 kg) anesthetized with either sodium pentobarbital (30 mg/kg intraperitoneally), or Dial with urethane (0.5 ml/kg intraperitoneally). One or 2 mg of atropine sulfate was administered intramuscularly or subcutaneously. Carotid blood pressure was recorded, by a constant perfusion technique, with a mercury manometer.[4] In some experiments nictitating membrane contractions were registered. Contractions produced by faradic stimulation of the preganglionic cervical sympathetic nerve were compared with drug-induced contractions.

Drug 194, in concentrations of about 2.5 x 10^{-3} M, was infused from a burette into a femoral vein during a period of about 20-60 minutes. Immediately before use, the drug was diluted from a stock solution of 0.1 M 194 in 95% alcohol (made 0.05 N acid with conc. H_2SO_4). The sympathomimetic amines were injected rapidly intravenously from a 1 or 2 ml tuberculin syringe. These were diluted from stock solutions containing 0.1% $Na_2S_2O_5$ and 0.5% chloretone. All stock solutions were kept in a refrigerator, and, with the exception of one solution of racemic norepinephrine, did not deteriorate. All drugs having an asymmetric carbon atom were used as racemic mixtures—with the exception of epinephrine, whose levo-isomer was used.

The type of experiment which could be performed depended upon the sympathomimetic amine to be used. Catecholic sympathomimetic amines do not cause tachyphylaxis,[5] so that repeated control doses were administered prior to the injection of the sympatholytic. A single dose of non-catecholic amine was injected after the pressor response to a test dose of 6 μg/kg of epinephrine had been reversed by 194. The dose of non-catecholic amine was chosen as that which had a pressor-potency equal to or greater than that of 6 μg/kg of epinephrine injected intravenously.

Results. I. Blood Pressure. *A. Catecholic pressor amines.* Since tachyphylaxis does not occur with multiple injections of these compounds, small doses (*i.e.* $\leqq$ 10 μ gm/kg)

* This investigation was supported by a grant from the Smith, Kline and French Laboratories.

1 Nickerson, M., and Goodman, L. S., *Proc. Am. Fed. Clin. Research*, 1945, **2**, 109.

2 Nickerson, M., and Goodman, L. S., *Fed. Proc.*, 1946, **5**, 194.

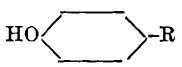
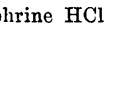
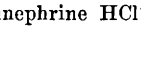
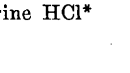
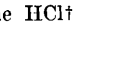
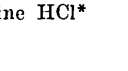
† This drug was furnished by the Smith, Kline and French Laboratories. It will be referred to as 194, the code number of the supplier.

3 Wells, J. A., and Rall, D. P., *Fed. Proc.*, 1948, **7**, 264.

4 Trendelenburg, P., *Pflüger's arch. f. d. ges. Physiol.*, 1924, **203**, 413.

5 Beyer, K. H., *Physiol. Revs.*, 1946, **26**, 169.

TABLE I.
Experiments with Catecholic Pressor Compounds.

Pressor compound	Formula  OH	No. of exp.	Dose range: Pressor cpd, μg/kg	Dose range: 194, μM/kg
Epinephrine HCl	R = 	63	0.5-8.0	10-77
Norepinephrine HCl*		23	0.5-6.0	10-77
Cobefrine HCl*		11	2.0-6.0	15-25
Epinine HCl†		2	6.0-16.0	15
Kephrine HCl*		2	2×10^3	15
Catechol	-H	2	$6\text{-}12 \times 10^3$	15

* Generously furnished by Dr. M. L. Tainter of the Sterling-Winthrop Research Institute.
† Generously furnished by Dr. E. J. de Beer of the Wellcome Research Institute.

were administered before and after the adrenolytic compound. Thus, effects of the ordinarily pressor compounds, epinephrine HCl, norepinephrine HCl (arterenol), cobefrine HCl, and epinine HCl, were compared in the pre- and post-sympatholytic periods. Table I summarizes these experiments.

When effects of a catecholic pressor amine in the pre- and post-sympatholytic periods were examined, the post-sympatholytic effect showed two principal types of difference from the pre-sympatholytic effect:

1) Abolition or reduction in magnitude of the initial pressor spike, and 2) revelation of a depressor action, when none had been present prior to the 194; or augmentation of a depressor effect, when, in the presympatholytic period, such effect followed the primary pressor spike.

In summary. a) Magnitudes of changes 1) and 2), undergone by a given sympathomimetic compound, tended to vary in the same direction; b) Magnitudes of changes 1) and 2) sustained by the animal after equal (by weight) small doses of the different sympathomimetic amines, in the presence of a given dose of 194, showed the following order: Epinephrine > norepinephrine ≥ cobefrine c) *Duration* of post-sympatholytic pressor or depressor effects usually varied directly with the *magnitude* of such effects (except with epinephrine, as explained below.)

In most experiments with epinephrine, pressor action of doses ≦ 8 μg/kg was completely or nearly completely abolished. In *every* experiment with epinephrine, impressive alterations, both of types 1) and 2), were seen. Pressor-depressor effects of epinephrine were modified by 194 in a manner quite similar to that reported for Dibenamine HCL:[6,7] nearly maximum depressor effects could be evinced with relatively small doses of epinephrine (2-6 μg/kg), and multiplying the dose by a factor as great as 10^3 produced only a prolongation of the depression, with little or no change in depth.

It is noteworthy that, in *every* experiment in which the 3 were compared, epinephrine consistently demonstrated substantially greater depressor activity, following the lytic agent, than did norepinephrine or cobefrine. Perceptible evidence of *any* depressor action was shown by small doses of norepinephrine

[6] Nickerson, M., and Goodman, L. S., *J. Pharm. Exp. Therap.*, 1947, **89**, 167.

[7] Biörck, G., *Acta physiol. Scand.*, 1947, **14**, 174.

and cobefrine in fewer than *half* the animals in which they were used.

Two experiments with epinine showed that alterations 1) and 2) were considerably smaller than with epinephrine. The primary pressor actions of kephrine (adrenalone) and catechol were abolished by prior infusion of 194 and were replaced by purely depressor responses. Since relatively large doses of these compounds must be used to evoke pressor effects, they were administered in the same manner as the non-catecholic pressor amines (see below). Tainter,[29] using ergotamine, also has achieved reversal of the pressor effect of catechol.

B. Non-catecholic pressor amines. In each experiment listed in Table II, the only injections were, in order: 1) infusion of sympatholytic compound; 2) one or two doses of 6 μg/kg of epinephrine (a dose which invariably produces a pressor response in the *absence* of the lytic agent) to confirm the presence of a "block" of the pressor action of epinephrine; and 3) a single dose of the sympathomimetic agent being examined (the possibility of tachyphylaxis was excluded by using a single dose of any amine except epinephrine).

The effect of each pressor amine, in the presence of the stated dose of 194, is shown in Table II. The data tabulated there demonstrate that the blood pressure elevation, usually observed after the intravenous injection of large doses of any of these compounds, has been greatly reduced or abolished. Furthermore, most of these compounds are shown to exert some depressor effect.

II. Nictitating membrane. In a series of 18 experiments, doses of 6-50 μM/kg of 194 (usually 15-20 μM/kg) were administered. Records were made of the nictitating membrane responses to faradic stimulation of the preganglionic (peripheral) stump of the homolateral cervical sympathetic trunk and to epinephrine injected into a femoral vein or into the homolateral carotid artery or both.

In general, contractions resulting from intravenous or intra-carotid epinephrine (1-8 μg/kg) were diminished considerably more, in a given animal, by a dose of 194, than were contractions of equal height resulting from standard faradic stimuli applied to the sympathetic nerve. In only 1 of 18 experiments did the 194 fail to reduce significantly the responses to epinephrine; in about one-third of these experiments, intravenous injection of 1-5 μg/kg of epinephrine was able to evoke *no* contraction, after 194 was infused. On the other hand, response to a standard faradic stimulus was altogether unaffected by 20-25 μM/kg of 194 in 3 of 10 experiments; and, in *no* instance, was a contraction resulting from adrenergic nerve stimulation completely prevented by 194. Moreover, sympatholysis, when present, developed much more slowly than adrenolysis. These results are similar to those reported for Dibenamine HCl.[8]

A "reversal" (*i.e.* relaxation in lieu of contraction) never occurred when epinephrine was administered after the lytic agent. Such "reversal" of epinephrine-effect was seen by Acheson (cit. by Morison and Lissak[9]) when he administered ergotoxine prior to the epinephrine injection. Small contractions (of nictitating membranes acutely denervated by transection of pre-ganglionic sympathetic fibres) sometimes produced by intravenous injection of doses of norepinephrine $\leqq 5$ μg/kg, were prevented by 15 μM/kg of 194. Similarly, larger contractions resulting from appropriate doses of propadrine or benzedrine were prevented or considerably diminished by the lytic compound. Moreover, the extremely prolonged contractions induced by suitable doses of benzedrine or desoxyephedrine could be interrupted by an infusion of 194 initiated *after* the contraction was well established.

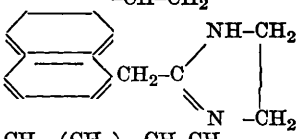
Discussion. The literature reveals reports of differences in behavior of sympathomimetic amines in the presence of different adrenolytic compounds. Table III summarizes some of these reports.

[29] Tainter, M. L., *J. Pharm. Exp. Therap.*, 1930, **40**, 43.

[8] Simeone, F. A., and Sarnoff, S. J., *Surgery*, 1947, **22**, 391.

[9] Morison, R. S., and Lissak, K., *Am. J. Physiol.*, 1938, **123**, 404.

TABLE II.
Experiments with Non-catecholic Pressor Compounds.

Name of pressor compound	Structural formula R_1	R_2	R_3	Dose of pressor cpd, mg/kg	Dose of 194, μM/kg	Effect after 194: Primary§ pressor, mm Hg	Effect after 194: Secondary‖ depressor, mm Hg
B-phenylethyl amine HCl*	H	H	$-CH_2-CH_2-NH_2$	1.31	15	0¶	14 (18)**
				1.30	15	0	12 (15)
Benzedrine SO_4	H	H	$-CH_2-CH(NH_2)-CH_3$	2.94	15	0	16 (18)
				2.94	15	0	18 (20)
Propadrine HCl*	H	H	$-CH(ON)-CH(NH_2)-CH_3$	1.80	15	0	10 (14)
				1.80	15	0	18 (20)
Desoxyephedrine HCl*	H	H	$-CH_2-CH(NHCH_3)-CH_3$	2.00	15	0	8 (7)
				2.00	15	6 (6)	16 (17)
				2.00	15	10 (17)	0
				1.98	15	0	?
Ephedrine SO_4	H	H	$-CH(OH)-CH(NHCH_3)-CH_3$	1.20	15	0	6 (7)
				1.20	19	0	14 (10)
Vonedrine HCl*	H	H	$-CH(CH_3)-CH_2-NHCH_3$	2.39	25	6 (5)	32 (27)
				2.38	25	8 (19)	6 (14)
Tyramine H_2PO_4	OH	H	$-CH_2-CH_2-NH_2$	0.91	15	0	14 (21)
				0.89	15	6 (8)	12 (15)
Paredrine HBr	OH	H	$-CH_2-CH(NH_2)-CH_3$	0.49	15	0	8 (11)
				0.49	15	0	0
Paredrinol SO_4*	OH	H	$-CH_2-CH(NHCH_3)-CH_3$	0.18	15	0	0
				0.36	25	6 (6)	0
Synephrine tartrate*	OH	H	$-CH(OH)-CH(NHCH_3)-CH_3$	0.74	17	0	10 (15)
				0.71	14	6 (6)	0
Neosynephrine HCl*	H	OH	$-CH(OH)-CH(NH-CH_3)-CH_3$	0.03	15	8 (9)	0
				0.03	15	6 (8)	0
Privine HCl ‡				0.06	25	6 (8)	0
				0.07	15	22 (32)	0
				0.06	25	8 (8)	0
Tuamine SO_4†	$CH_3-(CH_2)_4-CH(NH_2)-CH_3$			1.90	15	6 (10)	14 (23)
				1.90	15	0	22 (26)
				1.89	25	6 (5)	20 (18)
2-Amino-6 methyl heptane SO_4†	$CH_3-CH(CH_3)-(CH_2)_3-CH(NH_2)-CH_3$			1.00	16	0	16 (15)
				1.00	15	0	10 (9)
2-Amino-4-methyl hexane SO_4†	$CH_3-CH_2-CH(CH_3)-CH_2-CH(NH_2)-CH_3$			1.78	15	0	0
				1.83	15	0	8 (11)

* Generously furnished by the Smith, Kline, and French Laboratories.
† Generously furnished by Dr. K. K. Chen of the Lilly Research Laboratory.
‡ Generously furnished by Ciba Pharmaceutical Products, Inc.
§ Refers to initial pressor response which ordinarily follows intravenous injection of sympathomimetic compound.
‖ Refers to depressor effect following the diminished initial pressor response, or to the initial depressor effect when primary pressor effect has been abolished.
¶ "0" refers to a B.P. response $\leqq$ 4 mm Hg.
** Each transverse row of data is extracted from 1 experiment.
Quantities in parentheses are percentage changes, of pre-injection blood pressure, corresponding to absolute changes in mm Hg.

TABLE III.
Effects of Several Chemical Species of Adrenolytic Agents on Sympathomimetic Compounds.

Sympathomimetic pressor compound	Adrenolytic agent: Ergot alkaloids	Dioxane derivatives (Fourneau)	Priscol (benzyl-imidazoline)	N-Mustard derivatives	194*
Catechol	R[29]				R
Epinine	R[29]	R[15]			PR
Norepinephrine	B‡			R[3]	PR
Cobefrine	B‡			R[3]	PR
Kephrine	R[29]	R[15]		R[3]	R
B-phenylethylamine		B[15]			R
Benzedrine	B[7,12]	B[15]	B[16]	R[3,7]	R
Propadrine			B[16]	R[3]	R
Desoxyephedrine	NR[27]				R
Ephedrine	B†			O[17]	
		B[15]	B[16]		R
	R[10,11,12]			R[3]	
Vonedrine			B[16]		R
Tyramine	R[28]	B[15]	R[16]	R[3]	R
Paredrine			B[16]	R[3]	R
Paredrinol				R[3]	R
Synephrine	B[13]		B[16]		R
Neosynephrine	R[5]	R[15]	B[16]	R[3]	B
Privine			B[16]	R[3]	B
Aliphatic pressor compounds	O[14]		R[16]		R

O—No effect reported.
B—Partial or complete abolition of pressor effect; depressor activity not increased.
PR—Partial reversals: reduction or abolition of pressor effects consistently achieved; secondary depressor action sometimes produced or augmented.
NR—No reversals reported.
R—Complete or nearly complete reversals in some experiments.
* Experiments cited in present report.
† Controversy persists regarding reversal of ephedrine effect.
‡ In 2 experiments on cats, we found that 5 mg/kg of dihydroergocristine methane sulphonate and dihydroergokryptine methane sulphonate, respectively, partially or completely blocked pressor effects, but revealed no significant depressor action.
Numbers correspond to references in bibliography.

It is noteworthy that each of the pressor substances listed has demonstrated significant *depressor* activity under the influence of at least one lytic compound. It is likely that such depressor effects could be elicited in some instances (including our own experiments) in which only "blocking" of pressor properties has been reported, if modifications of experimental conditions, dosage, etc., were introduced.

In this connection, the influence of species of experimental animal is remarkable. For example, it has been shown that a variety of lytic agents (including ergot alkaloids,[18] dioxane derivatives,[15] and N-mustards[7] can *reverse* epinephrine-hypertension in

10 Barger, G., *Ergot and Ergotism*, London, 1931.

11 Goodman, L. S., and Gilman, A., *The Pharmacological Basis of Therapeutics*, New York, 1941.

12 Sollman, T., *A Manual of Pharmacology*, Philadelphia, 1947.

13 Tainter, M. L., and Seidenfeld, M. A., *J. Pharm. Exp. Therap.*, 1930, **40**, 23.

14 Ahlquist, R. P., *J. Pharm. Exp. Therap.*, 1945, **85**, 283.

15 Bovet, D., *J. Suisse Méd.*, 1943, **73**, 153.

16 Ahlquist, R. P., *et al.*, *J. Pharm. Exp. Therap.*, 1947, **89**, 271.

17 De Vleeschouwer, G. R., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 151.

18 Dale, H. H., *J. Physiol.*, 1906, **34**, 163.

27 Hauschild, F. (cit. by Haley, T. J., *J. Am. Pharm. Assn.*, 1947, **36**, 161).

28 Tainter, M. L., *J. Pharm. Exp. Therap.*, 1926-27, **30**, 163.

the cat but can only *prevent* it in the rabbit. This striking concordance of results may reflect a basic physiologic difference which can be explored for information related to the anatomical and biochemical foundations of sympatholytic activity and of the depressor effects of sympathomimetic substances.

Having found that paredrine reverses the pressor effect of vonedrine in the dog whereas priscol simply blocks it, Ahlquist[19] postulated that the former phenomenon represented an "actual reversal of action and not an unmasking of a normally present response." This interpretation hardly seems necessary, since we have demonstrated that the effects of vonedrine readily can be reversed by 194.

Hunt[23] has found that certain bis-β-chloroethyl derivatives of the N-mustard series block the blood-pressure changing effects of both *pressor and depressor* amines. If confirmed, this phenomenon would be unique since other compounds which have been shown to prevent certain inhibitory effects of epinephrine are unable to block the blood-pressure reducing action of epinephrine.[15,26]

Drug 194 is closely related chemically to Dibenamine HCl (dibenzyl-β-chloroethylamine HCl) and somewhat more remotely related to other N-mustard blocking agents concerning which publications have appeared recently.[3,20,21,22,23] Its effects, in our hands, were comparable to those of Dibenamine. However, the latter compound, as well as 194, usually caused a slow progressive fall in blood-pressure, sometimes to shock levels. Usually the fall in blood pressure terminated at a level of 60-100 mm of Hg. Fifteen μM/kg of 194 caused reversal of epinephrine (6 μg/kg) for at least 2-6 hours; whereas Biorck,[7] using comparable doses of epinephrine and Dibenamine which he injected rapidly (during 1-2 min.) found little or no adrenolysis after one hour.

The experiments reported here do not appear to contribute measurably to answering the problem of relating sympathomimetic chemical constitution, or vascular sites of action (where these are known), or both, to the capacity of a lytic substance to annul or reverse the effects of such sympathomimetic amines. The comprehensive ability of 194 to prevent certain pharmacodynamic effects of these sympathomimetic compounds does seem to support the concept of their eventual action, direct or indirect, on a point or points which are rendered inactive or inaccessible by reason of previous irreversible, or only slowly reversible, reaction with 194. ("Indirect" action of 'mimetic molecules is exemplified by the postulate of Gaddum and Kwiatowski[24] regarding the mode of action of ephedrine.) The work of Bergmann *et al.*[25] supports the possibility that 194 (and other N-mustard blocking agents) might affect more than one reactive "receptor" group.

Summary and Conclusions. 1. Drug 194 (N-benzyl-N-β-phenylisopropyl-β chloroethylamine hydrochloride), has been shown to be a potent antagonist of certain excitatory effects of a number of sympathomimetic agents. In the cat, 194 reduced or abolished pressor responses to aliphatic and aromatic amines and to catechol. In the presence of 194, all the compounds, except neosynephrine, privine, and paredrinol, manifested depressor activity.

2. The effects of nerve stimulation on the nictitating membrane of the cat were less effectively blocked by 194 than were the effects of epinephrine. 194 also could prevent or interrupt nictitating membrane contractions induced by sympathomimetic amines other than epinephrine.

19 Ahlquist, R. P., *J. Am. Pharm. Assn.*, 1946, **35**, 348.

20 Achenbach, P., and Loew, E. R., *Fed. Proc.*, 1947, **6**, 304.

21 Loew, E. R., and Micetich, A., *Fed. Proc.*, 1947, **6**, 351.

22 Loew, E. R., Micetich, A., and Achenbach, P., *Fed. Proc.*, 1947, **6**, 351.

23 Hunt, C. C., *Fed. Proc.*, 1948, **7**, 229.

24 Gaddum, J. H., and Kwiatowski, L., *J. Physiol.*, 1938, **94**, 87.

25 Fruton, J. S., Stein, W. H., and Bergmann, M., *J. Org. Chem.*, 1946, **11**, 559.

26 Rothlin, E., *Bull. Acad. Suisse Sci. Med.*, 1946-47, **2**, 1.