SYMPATHOMIMETIC ACTIONS OF *CIS*-2-AMINO-4-METHYL-5-PHENYL-2-OXAZOLINE

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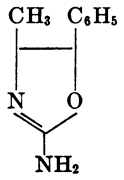
In 1958 Burn and Rand suggested that sympathomimetic drugs may be divided into two classes: (a) substances that produce sympathomimetic effects in both normal and reserpine pretreated animals and (b) substances that produce sympathomimetic effects in normal animals but have little or no effect in reserpine pretreated animals. Since one of the effects of reserpine is a depletion of catecholamines from tissue stores, these authors postulated that sympathomimetic drugs that failed to cause a pressor effect in the reserpinized animal normally acted by causing a release of catecholamines from tissues. The observation that the pressor action of certain sympathomimetic amines can be restored in the reserpine treated animals by an infusion of norepinephrine is cited as support for the concept of indirect acting sympathomimetic agents.

The effects of compounds belonging to either of these two classes are not antagonized by ganglionic blocking drugs; therefore ganglionic stimulants such as nicotine, DMPP (1,1-dimethyl-4-phenylpiperazinium iodide) and McN-A-343, a new ganglionic stimulant described by Roszkowski (1961) (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride) are excluded from this classification.

cis-2-Amino-4-methyl-5-phenyl-2-oxazoline (McN-822), although structurally different from the usual type of sympathomimetic agent, was found to produce sympathomimetic cardiovascular effects in dogs. The chemistry of this compound has been described by Poos *et al.* (1963). This paper is a report of a study undertaken to determine whether McN-822 belongs to either of the two classes of sympathomimetic drugs.



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METHODS AND MATERIALS. Mongrel dogs of either sex, anesthetized with sodium pentobarbital (30 mg/kg, i.v.), were used in this study. Blood pressure was obtained from a carotid artery by means of a mercury manometer and recorded on a kymograph, except in tests in open-chest dogs in which simultaneous recordings of myocardial contractile force and blood pressure were made. In these tests the heart was exposed by an intercostal incision; the pericardium was slit and a Walton strain gauge arch was sutured to the surface of the right ventricle (Boniface *et al.*, 1953). Blood pressure was measured from a carotid artery connected to a Sanborn pressure transducer. Recordings of blood pressure and contractile force were made on a Sanborn Model 150 recorder. The dogs were artificially ventilated with a Palmer pump. Unless otherwise stated, all injections were made into a cannulated femoral vein.

In experiments in which it was desirable to use dogs depleted of catecholamines, animals that had been given reserpine (0.5 mg/kg, i.p.) once a day for 2 consecutive days were used (Burn and Rand, 1958). The experiments were carried out on the third day.

Statistical analysis of the data was made according to methods presented by Mainland (1952).

Aqueous solutions of the following compounds were used: *l*-epinephrine and *dl*-isopropylnorepinephrine (isoproterenol) as the bitartrates, hexamethonium chloride, pentolinium tartrate, dichloroisoproterenol hydrochloride (DCI), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), *d*-amphetamine sulfate, atropine sulfate, and phenoxybenzamine hydrochloride. The doses of these compounds are expressed in terms of the salt and the doses of the following compounds are expressed in terms of the base. Dibozane (1,4-bis-(1,4-benzodioxan-2-ylmethyl)piperazine) was dissolved in 0.8% phosphoric acid, and reserpine was prepared either in ethanol and glacial acetic acid or in 20% ascorbic acid. McN-822 was dissolved in 1.5 equivalents of 1 N HCl and diluted to a 2% solution with distilled water.

RESULTS. The sympathomimetic effects of McN-822 were manifested in dogs by an increase

in blood pressure, heart rate and contractile force.

A mean increase in blood pressure of 17% was obtained in 4 dogs following an initial injection of 0.25 mg/kg of the compound. A second injection of 0.5 mg/kg given 10 to 30 minutes later had no effect in 1 dog and caused an average increase in blood pressure of 15% in 3 dogs. Subsequent injections of 1, 2 and 4 mg/kg at intervals of 5 to 20 minutes produced predominantly transient depressor responses.

The first injection of 1 or 4 mg/kg of McN-822 produced an average increase in blood pressure of 40% in 4 dogs and 45% in 3 dogs, respectively. There was little or no pressor response when the same doses were repeated in 30 to 60 minutes, while epinephrine produced its usual marked increase in blood pressure (fig. 1). The magnitude of the pressor response caused by a dose of 4 mg/kg of McN-822 was not as great as that caused by 1 μg/kg of epinephrine; however, the duration of the response to McN-822 was approximately 10 to 20 times greater.

The stimulant effects of McN-822 on heart rate and contractile force in vagotomized dogs are shown in table 1. A dose of 1 μg/kg of epinephrine produced greater responses than 0.25 mg/kg of McN-822.

The pressor effects of McN-822 were potentiated in 4 dogs pretreated with hexamethonium or pentolinium (2 mg/kg). In these tests a dose of 0.25 mg/kg of McN-822, given approximately 10 minutes after the ganglionic blocking agents, caused an average increase in blood pressure of 78% as compared with 17% in control dogs. The ability of the blocking agents to abolish or markedly reduce the pressor response to DMPP (15 to 30 μg/kg) was taken as evidence of effective ganglionic blockade.

Typical pressor responses were obtained in atropinized dogs (0.5 to 1 mg/kg) following doses of 0.25 mg/kg (3 dogs) and 4 mg/kg (3 dogs) of McN-822. The doses of atropine used were shown to abolish the depressor effects of 5 μg/kg of acetylcholine.

Phenoxybenzamine (10 mg/kg) completely blocked or markedly reduced the hypertensive effects of McN-822 (table 2). A period of $1\frac{1}{2}$ hours was allowed for phenoxybenzamine to produce effective adrenergic blockade characterized by reversal of the pressor response to epinephrine (2 μg/kg).

McN-822, as shown in table 3, failed to increase the heart rate and had little or no effect on myocardial force of contractions in 4 dogs pretreated with DCI (5 mg/kg), a specific blocker of the effects of sympathomimetic agents on the heart (Moran and Perkins, 1958). It was assumed that an effective blockade existed because isoproterenol (0.5 μg/kg) failed to produce its usual marked stimulant effects on the heart after the injection of DCI.

The ability of McN-822 (1 mg/kg) to cause an increase in blood pressure was significantly reduced ($P < .01$) in 6 dogs pretreated for 2 days with reserpine (0.5 mg/kg, i.p.), while the pressor responses to epinephrine (1 to 2 μg/kg) appeared to be potentiated.

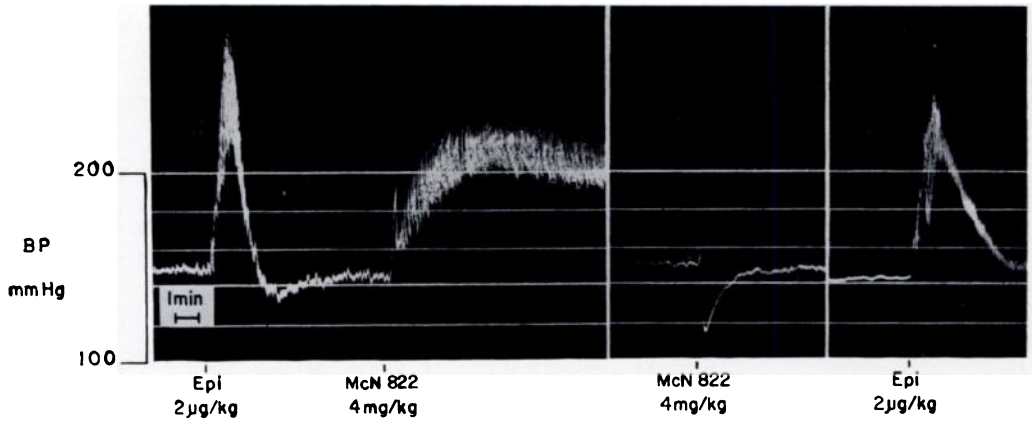

FIG. 1. Effects of McN-822 on arterial blood pressure in the anesthetized dog.
The pressor response to McN-822 lasted about 40 minutes. A second injection of McN-822 was given 60 minutes following the first dose; tachyphylaxis is shown. The ability of the dog to respond to a sympathomimetic is evident by the pressor effect obtained with epinephrine given 15 minutes after the second injection of McN-822.

TABLE 1

Effects of McN-822 on heart rate and contractile force in vagotomized dogs

Dog No.	Dose	% Increase	
		Heart rate	Contractile force
	mg/kg, i.v.		
1	0.25	21	62
	0.5	19	40
	1.0	11	15
	2.0	6	0
2	0.25	22	67
	0.5	20	38
	1.0	21	30
	2.0	0	13, 7[a]
3	0.25	22	76
	0.5	16	48
	1.0	11	14
	2.0	5	5

[a] 13% decrease in contractile force.

TABLE 2

Comparison of blood pressure responses to McN-822 (1 mg/kg, i.v.) in control dogs and dogs pretreated with phenoxybenzamine

Dog No.	% Change in Blood Pressure
Controls	
1	37 inc.
2	39 inc.
3	36 inc.
4	50 inc.
Pretreated with phenoxybenzamine (10 mg/kg, i.v.)	
5	8 inc.[a] 19 dec.
6	12 dec.[a] 12 inc.
7	18 dec.[a] 13 inc.
8	15 inc.

[a] Indicates biphasic response.

Cross tachyphylaxis was demonstrated between McN-822 and amphetamine. Three dogs were given repeated injections of amphetamine (1 mg/kg), until the compound failed to produce a pressor effect; this occurred after the second or third injection. McN-822 (1 mg/kg), given approximately 10 minutes after the development of tachyphylaxis to amphetamine, failed to produce a pressor response. Conversely, amphetamine (1 mg/kg), given approximately 10 minutes after tachyphylaxis to McN-822 had developed, did not cause its typical pressor effect in 4 dogs.

TABLE 3

Comparison of heart rate and contractile force effects of McN-822 (0.5 mg/kg) in control dogs and dogs pretreated with DCI

Dog No.	% Increase	
	Heart rate	Contractile force
Controls		
1	43	71
2	23	65
3	21	70
4	62	29
Mean	37	59
Pretreated with DCI (5 mg/kg, i.v.)		
5	0	10
6	0	0
7	0	0
8	0	2
Mean	0	3

The usual slight depressor response to an acute injection of reserpine (1 mg/kg), given 5 to 10 minutes after an initial dose of McN-822 (0.1 mg/kg), was reversed in 4 dogs (fig. 2). Likewise, using the same doses and time intervals between doses indicated above, an initial injection of reserpine caused marked potentiation of the pressor effect of McN-822 (fig. 3). These pressor responses were readily antagonized by dibozane, a potent adrenergic blocking agent (O'Leary, 1953) and did not occur in 6 dogs pretreated with reserpine for 2 days. Typical reserpine pressor responses were obtained in 4 dogs given a dose of 0.05 mg/kg of McN-822 ten minutes before reserpine (1 mg/kg) or when reserpine was injected immediately after McN-822 (3 dogs).

Tests were carried out in 14 atropinized (1 mg/kg) dogs to determine the effects of McN-822 on the pressor responses to epinephrine or norepinephrine. Control responses to 2 injections each of 0.5 and 1 μg/kg of epinephrine (7 dogs) or norepinephrine (7 dogs) were compared with the responses obtained 10 minutes after the injection of 0.05 or 0.1 mg/kg of McN-822. The changes in the intensity and duration of the responses to both doses of norepinephrine and

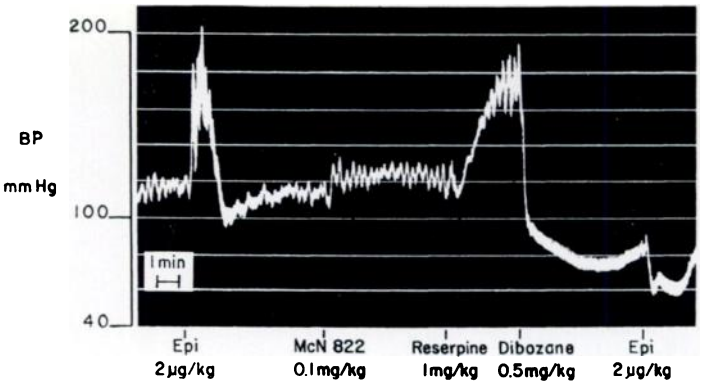

FIG. 2. Pressor effect of reserpine in an anesthetized dog pretreated with McN-822 5 minutes before the injection of reserpine.

The ability of dibozane, an adrenergic blocking agent, to terminate the pressor effect of reserpine and reverse the epinephrine response, is shown.

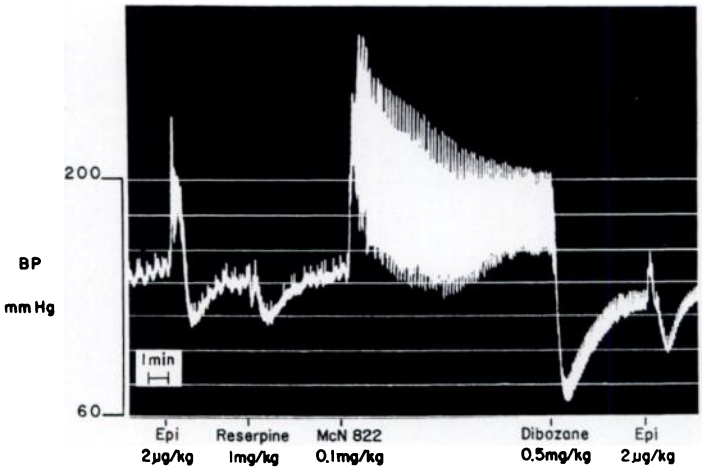

FIG. 3. Marked potentiation of the pressor effect of McN-822 in an anesthetized dog given reserpine 5 minutes before the injection of McN-822.

Antagonism of the blood pressure effects of McN-822 and epinephrine by dibozane, an adrenergic blocking agent, is shown.

the lower dose of epinephrine were slight, variable and considered to be insignificant. The intensity of the responses to epinephrine (1 µg/kg) was increased 9 to 29% (mean 19%) and 4 to 26% (mean 16%) following McN-822 (0.05 and 0.1 mg/kg). There was either no change or an increase (maximum, 30 seconds) in the duration of the epinephrine responses. Reserpine given at the end of each experiment produced a marked pressor effect. There was no apparent relationship between the intensity of the reserpine response and the possible potentiation of epinephrine.

DISCUSSION. The pressor and cardiac stimulant actions of McN-822 are considered to be sympathomimetic in nature since the pressor effects can be antagonized by phenoxybenzamine and the cardiac effects can be antagonized by DCI. Repeated injections of the compound result in a transition from a pressor response to a transient depressor response. This is not due to an inability of the cardiovascular system to respond to sympathomimetic agents, since epinephrine is still capable of producing its usual pressor effect; consequently, it is concluded that tachyphylaxis develops to McN-822.

The possibility that McN-822 produces its effects by an action on sympathetic ganglia was eliminated in part by the demonstration that the pressor effect of McN-822 is not antagonized but

is, in fact, potentiated in dogs pretreated with ganglionic blocking drugs. The ability of McN-822 to produce typical pressor effects in atropinized dogs indicates McN-822 does not exert a ganglionic stimulant effect in the manner of McN-A-343, whose stimulant effects are abolished by small doses of atropine (Roszkowski, 1961).

The relative inability of McN-822 to cause an increase in blood pressure in dogs presumably depleted of catecholamines suggests that the sympathomimetic effects of McN-822 are mediated indirectly by released catecholamines. In this respect McN-822 is like amphetamine and tyramine (Burn and Rand, 1958).

The interesting interactions between reserpine and McN-822, resulting in marked pressor responses, are believed to be due to released catecholamines since the responses can be antagonized by an adrenergic blocking drug like dibozane and do not occur in dogs presumably depleted of catecholamines.

The lack of potentiation of norepinephrine and the questionable potentiation of epinephrine suggest that increased sensitivity to these amines or interference with the metabolism of these amines is not a primary factor in the pressor responses resulting from an interaction of reserpine and McN-822.

One explanation of the pressor responses resulting from an interaction of reserpine and McN-822 is that the rate of release of catecholamines is greater under the combined influence of these agents than it is under the influence of either agent alone. A similar explanation was offered by Schmitt and Schmitt (1961) to explain the pressor effect of reserpine following an injection of amphetamine.

SUMMARY

The results of this study indicate that McN-822 belongs to a class of sympathomimetic agents that produce effects in normal dogs and have little or no effect in dogs presumably depleted of catecholamines by reserpine. This suggests McN-822 produces its sympathomimetic effects indirectly through the release of catecholamines. The marked sympathomimetic effects resulting from injections of reserpine and McN-822 in normal dogs are believed to be due to an enhanced release of catecholamines under the combined influence of both compounds.

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