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THE EFFECTS OF N-METHYLATION ON THE PHARMACOLOGICAL ACTIVITY OF PHENETHYLAMINE (1)

BY

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The biologically important catecholamines are derivatives of phenethylamine. The formation of catecholamines by the addition of phenolic and alcoholic hydroxyl groups to phenethylamine results in various activities. Numerous studies have been reported on the significance of these groups (1, 2). However, insufficient attention has been afforded the effects of N-methylation of adrenergic amines.

Numerous investigators have studied the adrenergic activity of phenethylamine (1, 2). Others have classified phenetylamine as being of the indirect (catecholamine releasing) type (3, 4). SCHMIDT and FLEMING (5) concluded that phenethylamine possessed both direct and indirect actions. ZAMBONI (6) had found that N, N-dimethylphenethylamine produced a depressor response in the dog. GADDUM *et al.* (7) in a study of N-substituted catecholamines reported that N-methyl epinephrine was 1/250 as active as epinephrine on the nictitating membrane of the cat. HUME and HOLLAND (8) had observed that N, N, N-trimethylphenethylamine possessed a nicotine-like stimulant action.

Thus, it is the purpose of this investigation to study the relationship of N-substituted methyl groups in a series of phenethylamines to stimulant activity on the autonomic nervous system.

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Materials and Methods

The series of compounds were studied on the vasopressor response in the dog and spinal cat, contraction of the nictitating membrane of the cat following injection of the compounds to the membrane and to the superior cervical ganglion via the lingual artery.

Blood pressure measurements. — Mongrel dogs of both sexes weighing between 8–15 kg were used. The dogs were anesthetized with 30 mg/kg sodium pentobarbital i.v.; anesthesia was maintained with 3 mg/kg as needed. Cats of both sexes weighing between 2–4 kg were used. After anesthesia was induced with ether, spinal preparations were made as described by Burn (3). Changes in blood pressure were recorded via the cannulated left carotid artery with a mercury manometer and recorded on smoked paper. The right femoral vein was cannulated for injection of drugs. The drugs were dissolved in normal saline for injection.

Nictitating membrane. — Cats of either sex weighing 2–4 kg were used. Spinal cats were prepared according to a method described by Burn (3) and modified by Trendelenburg (4) in that drugs could be selectively injected by way of the lingual artery directly to the nictitating membrane or by clamping off the external carotid, the drug could be directed to the superior cervical ganglion. This preparation is suitable for the separation of adrenergic and cholinergic activities of the compounds under study. The movement of the nictitating membrane were recorded by means of a Grass Polygraph. The tension on the nictitating membrane was either 2 or 7 gm. The sensitivity was set so that one cm was equal to one gram of tension.

For preganglionic stimulation, the vagus nerve was isolated and severed. One end was placed on a bipolar platinum electrode and insulated with liquid petrolatum. An electronic stimulator producing rectangular impulses of 1 msec duration was used. Stimulation of supramaximal strength was applied in all experiments, that is, 2–4 v and at a frequency of 25 cps. The drugs were injected first to the nictitating membrane and then to the superior cervical ganglion. The membrane was allowed to return to pre-injection level for 5 minutes before the next injection.

For calibration, epinephrine (1.25 mcg/kg) was injected to the nictitating membrane and acetylcholine (30 mcg/kg) was injected to the superior cervical ganglion.

The following drugs were prepared on an equal molar basis using N, N, N-trimethylphenethylamine as the standard: Phenethylamine, N-methylphenethylamine, N, N-dimethylphenethylamine. The volume of injection to the nictitating membrane was 0.2 ml of the solution of the drug, followed by 0.2 ml of normal saline.

Chemical synthesis. Phenethylamine was obtained from Eastman Organic Chemicals, N-methylphenethylamine from Aldrich Chemical, N, N-dimethylphenethylamine was prepared according to Icke and Wisegarver (9). N, N, N-trimethylphenethylamine was prepared by allowing N, N-dimethylphenethylamine to react with methyl iodide in ether. The produce was recrystallized from an ether-ethanol mixture to a constant melting point. The hydrochlorides of phenethylamine, N-methylphenethylamine and N, N-dimethylphenethylamine were prepared by passing anhydrous HCl through a solution of the amine in ether, filtering off the resulting precipitate and recrystallizing from an ethanol solution.

Infrared and nuclear magnetic resonance spectra were obtained to confirm structure and purity of the compounds used in this study.

Results

The data from the experiments are summarized in Fig. 1 and Table I. Typical responses of the nictitating membrane to (A) stimulus, 4 v; (B) Phenethylamine, 372 mcg/kg; (C) N-methylphenethylamine, 372 mcg/kg; (D) N, N-dimethylphenethylamine, 555 mcg/kg injected directly into the membrane are shown in Fig. 1. From Table I, phenethylamine

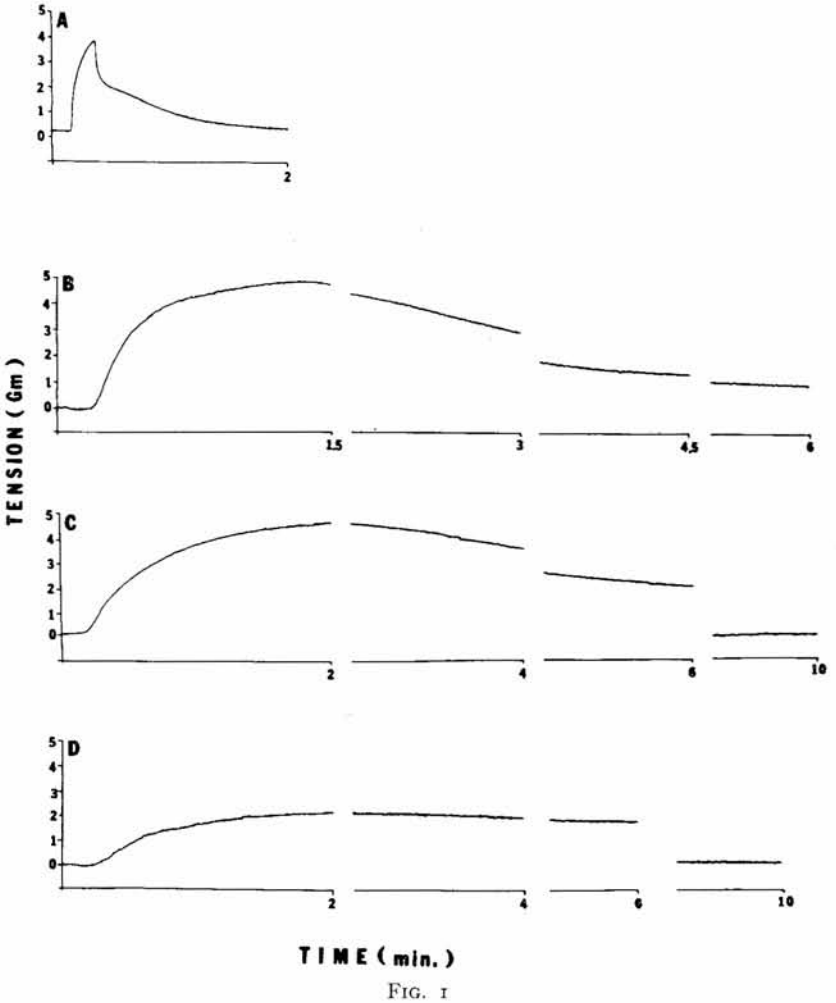

Fig. 1

Typical responses of the nictitating membrane of the cat to N substituted phenethylamines. (A) stimulus, 4 v; (B) Phenethylamine, 372 mcg/kg; (C) N-methylphenethylamine, 372 mcg/kg; (D) N, N-dimethylphenethylamine, 555 mcg/kg.

TABLE I

Relative vasopressor activity of the dog and cat, and response of the nictitating membrane of the cat to N substituted phenethylamines, molar basis ± S.D.

Compound	Name	Relative Activities Molar Basis			
		Nictitating Membrane Cat	Superior Cervical Ganglion Cat	Cat Blood Pressure	Dog Blood Pressure
$C_6H_5(CH_2)_2NH_2$	Phenethylamine	1.00	—	1.00	1.00
$C_6H_5(CH_2)_2NCH_3$	N-methylphenethylamine	0.80 ± 0.03	0.02 ± 0.0014	1.13 ± 0.06	0.71 ± 0.14
$C_6H_5(CH_2)_2N(CH_3)_2$	N,N-dimethylphenethylamine	0.38 ± 0.15	0.07 ± 0.0015	0.38 ± 0.04	0.09 ± 0.02
$C_6H_5(CH_2)_2N^+(CH_3)_3$	N,N,N-trimethylphenethylamine	—	1.00	0.88 ± 0.08	0.89 ± 0.16

when injected directly to the membrane produced the greatest response of the nictitating membrane. The addition of one methyl group (N-methylphenethylamine) reduced the adrenergic response approximately one fifth, and with the addition of a second methyl group (N, N-dimethylphenethylamine) the adrenergic activity is further decreased. In the case of three methyl groups (N, N, N-trimethylphenethylamine) which form a quaternary ammonium compound, adrenergic activity was abolished.

Only N, N, N-trimethylphenethylamine ellicited a significant response when injected to the superior cervical ganglion.

At a dose level of 40 mcg/kg the order of activity of response of blood pressure elevation of the cat was : $—NCH_3 > —NH_2 > —N(CH_3)_3 > —N(CH_3)_2$; however, at a dose of 80 mcg/kg the order of activity was observed to be : $—N(CH_3)_3 > —NCH_3 > —NH_2 > —N(CH_3)_2$. These orders of relative activity were unchanged following the atropinization (1 mg/kg) of the animal.

Following treatment with reserpine, injected intraperitoneally, 24 hours prior to the experiments, a significant reduction was observed in response of the nictitating membrane to the drugs employed. There was also a marked reduction in response to preganglionic stimulation. Epinephrine, injected to the nictitating membrane, continued to produce a maximal response.

The response of the drugs injected to the nictitating membrane was abolished after the intravenous injection of phentolamine (5 mg/kg).

Atropine (1 mg/kg) had no effect on the activity of these drugs in producing contraction of the nictitating membrane.

Discussion

From these experiments, the pharmacologic activity of N-methyl substituted phenethylamines progresses from an indirect adrenergic activity with phenethylamine to a purely cholinergic activity of the nicotinic type with N, N, N-trimethylphenethylamine. This change in activity is apparently the result of the substitution of methyl groups on the nitrogen atom.

Trendelenburg *et al.* (4) have observed that the replacement of the N-methyl group with a hydrogen atom reduced the potency of several "direct" sympathomimetic amines; however, N-methylation of dopamine resulted in an increased direct activity, according to Palm *et al.* (10). Our data indicate that the replacement of the N-methyl group with a hydrogen atom increases the potency of compounds of the indirect type of adrenergic activity.

It has been proposed by SCHUMANN and PHILLIPPU (11) that the indirect adrenergic compounds exert their effects by uptake into the presynaptic nerve terminals to displace catecholamines, which are released to produce the observed responses. It seems possible that the addition of methyl groups on the nitrogen could produce a hinderance to this uptake and in the case of N, N, N-trimethylphenethylamine, there is no uptake at all and, therefore, no response. This compound, however, exhibits cholinergic (nicotinic) activity in producing stimulation of the superior cervical ganglion by interacting with the cholinergic receptor in a manner similar to acetylcholine. An interaction between the cationic quaternary ammonium group of cholinergic agents and the anionic site of the cholinergic receptor has been proposed by WASER (12) and BARLOW (13).

THOMAS and MARLOW (14) had suggested that the positive charge on the nitrogen is equally shared with each of the methyl groups of the quaternary ammonium group rather than entirely upon the nitrogen atom itself. If the interaction of the phenethylamines and receptor depended solely upon the positive charge on the nitrogen atom itself, phenethylamine would possess the greatest cholinergic activity because the positive charge on the unsubstituted nitrogen atom would be much greater than that of methyl substituted nitrogen. From the data presented, it exhibited no cholinergic activity, but, did produce the greatest adrenergic response. Therefore, it can be concluded that the methyl groups, not the positive charge on the nitrogen alone are essential to drug-cholinergic receptor interaction.

After reserpinization 24 hours prior to the experiments, the observed reduction in activity of these compounds indicated the action of these agents was due to a release of catecholamines from their storage site at the adrenergic nerve endings and not from direct action on the nictitating membrane. However, the relative order of activities were the same as in the non-reserpinized animals.

Abolishment of response of the nictitating membrane by the blocking agent, phentolamine, would indicate that the alpha adrenergic receptor is involved in the adrenergic response of these compounds.

Since acetylcholine is recognized as possessing a muscarinic type of activity on the nictitating membrane, it could be proposed that the response of the membrane ellicited by phenethylamine, N-methylphenethylamine and N, N-dimethylphenethylamine could also be muscarinic. However, the administration of atropine did not block the activity of these compounds as it did acetylcholine. N, N, N-trimethylphenethylamine has been observed by HUNT (15) and HUME and HOLLAND (8) to produce only a nicotinic type of cholinergic response and, therefore,

would not be expected to stimulate the nictitating membrane when injected directly.

It is interesting to note that the effects on the cardiovascular system produced by the "nicotinic" properties of the N, N, N-trimethylphenethylamine are not as great as the equimolar doses of indirect adrenergic phenethylamines and its N-methyl derivates at a dose level of 40 mcg/kg. However, at a dose level of 80 mcg/kg, the response to the quaternary ammonium derivative is 50 % more than that of the other compounds. This is probably due to a difference in dose-response relationships of the ganglionic stimulant trimethyl compound and the adrenergic stimulants.

PALM *et al.* (10) have reported that the N-methyl derivative of dopamine possessed a greater affinity for the adrenergic beta receptors and a direct type of alpha adrenergic activity. These authors attributed their alterations in activity to the +I effect on the nitrogen atom of the methyl group. This would result in enhancement of the protonation of the nitrogen. However, from our data, it appears that the addition of one N-methyl group decreased indirect adrenergic activity somewhat, but the addition of a second N-methyl group decreased this activity greatly. These results do correlate with the ability of amines to form adducts with Lewis acids such as trialkylborons, which are electron acceptors (16).

SUMMARY

The effects of replacement of hydrogen atoms with N-methyl groups in phenethylamine have been studied on the blood pressure of cat and dog, and the nictitating membrane and superior cervical ganglion of the cat. The order of relative activity in response of the nictitating membrane was found to be : $—NH_2 > —NCH_3 > —N(CH_3)_2$. N, N, N-trimethylphenethylamine produces no effect on the nictitating membrane. The order of relative activity of response of blood pressure elevation of the cat to this series at a dose level of 40 mcg/kg was : $—NCH_3 > —NH_2 > —N(CH_3)_3 > —N(CH_3)_2$.

It appears that N-methyl substitution, in the absence of phenolic and/or alcoholic hydroxyl groups does not enhance alpha or beta activity nor increase the "direct" activity of the adrenergic-like compounds.

These results are correlated with the ability of amines to form adducts with Lewis acids.

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