Modification of Epinephrine-Induced Inhibition of Transmission by Tropolone and Desipramine (Desmethylimipramine) in the Perfused Superior Cervical Ganglion

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Abstract □ Tropolone, an inhibitor of catechol-*O*-methyltransferase (COMT), and desipramine (desmethylimipramine, DMI), which impairs catecholamine uptake at presynaptic adrenergic membranes, were introduced into the superior cervical ganglion of the cat. Throughout a wide dosage range (3.12–800 mcg.) tropolone exerted a negligible effect on epinephrine-induced inhibition of ganglionic transmission. COMT does not appear, therefore, to participate to a significant extent in peripheral ganglionic inactivation of circulating epinephrine. DMI in doses (125 and 250 mcg.) which alone had no effect on postganglionic potentials facilitated the depression of ganglionic transmission by epinephrine. Potentiation of epinephrine activity by DMI is attributed to a decrease in catecholamine uptake capacity at ganglionic sites. Catecholamine uptake thus appears to be a factor in terminating the activity of circulating epinephrine at sympathetic ganglia. Evidence for the existence in the ganglion of an uptake mechanism lends support to the proposal that catecholamines may function in the regulation of peripheral sympathetic ganglionic transmission.

Keyphrases □ Epinephrine inhibition—ganglionic transmission □ Tropolone, desmethylimipramine effect—epinephrine activity □ Cervical ganglion perfusion—tropolone, desmethylimipramine, epinephrine □ Catecholamine uptake—epinephrine activity

Considerable evidence suggests the participation of an adrenergic inhibitory system in peripheral ganglionic transmission (1–6). Based on this concept it might be anticipated that pharmacologic blockade of pathways of catecholamine inactivation would facilitate the response of ganglion cells to these amines.

Goldberg and DaCosta (7) and Gertner (8) reported that various monoamineoxidase (MAO) inhibitors effectively blocked sympathetic ganglionic transmission. However, Urquiaga *et al.* (9) found no correlation between the potency of the MAO inhibitor and the extent of reduction of postganglionic spike potentials. Other studies (10, 11) found ganglionic transmission to be unaffected by the administration of MAO inhibitors. The relevance of these observations to the functioning of an adrenergic inhibitory mechanism in ganglionic transmission is questionable in view of the limited role of MAO in the extraneuronal inactivation of catecholamines (12).

Oxygen methylation and uptake by adrenergic nerve terminals constitute major inactivating mechanisms for circulating and locally released catecholamines [*cf.* references cited by Axelrod (13)]. The participation of these systems in the termination of epinephrine activity within sympathetic ganglia was evaluated by the utilization of tropolone as an inhibitor of catechol-*O*-methyl transferase (COMT), and desipramine[1] (desmethylimipramine, DMI) as an inhibitor of the neural uptake of catecholamines.

[1] Desipramine is the United States Adopted Name for desmethylimipramine.

EXPERIMENTAL

The 36 cats (1.9–3.8 kg.) utilized in this study were anesthetized with urethan (1.2 g./kg., i.p.) and prepared for arterial perfusion of the superior cervical ganglion and monitoring of evoked postganglionic potentials according to the procedures detailed by Volle and Koelle (14) and Giller *et al.* (15). The perfusion fluid (16) which had a pH of approximately 7.3, was warmed to 37°, saturated with 100% O_2, and introduced at a constant rate (0.39 ml./min.) into the ganglion circuit *via* the common carotid artery.

Rectangular pulses of 0.1-msec. duration and supramaximal intensity were applied *via* platinum electrodes to the preganglionic fibers (cervical sympathetic trunk) at a rate of 0.5 shock/sec. Evoked postganglionic potentials from the external carotid nerve were amplified using a capacitance-coupled preamplifier[2] coupled to a dual-beam oscilloscope.[3] Permanent recordings were made on photographic paper[4] using an oscillotron.[5]

All drugs were dissolved in 0.9% sodium chloride and were introduced into the ganglion perfusion system in volumes not greater than 0.2 ml. *l*-Epinephrine bitartrate was administered at 10-min. intervals to determine for each preparation the dose capable of inducing an approximate 50% reduction of the postganglionic potential. The potential was recorded at the point of maximum reduction (20–50 sec.), and 60, 80, and 100 sec. after injection of epinephrine. Following establishment of control levels of response, various doses of tropolone or DMI were administered. The influence of these compounds on the ganglionic inhibitory effect of epinephrine was determined at 5, 15, 30, 60, 90, 120, 150, and 180 min. and compared to control. Data are reported in terms of maximum percent inhibition.

RESULTS

Effect of Tropolone on Epinephrine-Induced Inhibition of Ganglionic Transmission—Preliminary investigation established that close arterial injection of relatively large doses of tropolone (1.6 and 3.2 mg.) produced marked reduction of postganglionic potentials. Doses of this magnitude were, therefore, not suitable for study of the possible effects of COMT-inhibition on epinephrine depression of ganglionic transmission. However, doses of tropolone on the order of 0.8 mg. or less produced no discernible effect on postganglionic potentials during the 3-hr. period after administration.

The dose of epinephrine which evoked an approximate 50% reduction of the postganglionic action potential was administered at predetermined intervals during the 3-hr. period after injection of 3.12, 25, 200, 400, and 800 mcg. of tropolone. With the exception of a greater degree of depression of ganglionic transmission 90 and 120 min. after administration of 25 mcg. of tropolone (Table I), no statistically significant potentiation or prolongation of the inhibitory effect of epinephrine was observed (Table II).

Effect of DMI on Epinephrine-Induced Inhibition of Ganglionic Transmission—Close arterial administration of relatively large doses of DMI abolished the postganglionic potential; blockade of transmission persisted approximately 20 min. after 500 mcg. and 90 min. after 2,000 mcg. of DMI. A similar blockade of transmission in the superior cervical ganglion of the cat was observed by Urquiaga

[2] Tektronix RM122.
[3] Tektronix 502.
[4] Polaroid, type 47.
[5] Beattie, type 12666.

Table I—Effect of Close Arterial Injection of Tropolone on Epinephrine-Induced Inhibition of Postganglionic Potentials

Min.	Control	Tropolone, mcg. 3.12	Tropolone, mcg. 25
−5[a]	48.0 ± 1.5[b]	50.7 ± 2.9	48.4 ± 2.6
5	46.5 ± 2.2	52.0 ± 3.0	48.2 ± 1.1
15	44.9 ± 2.1	50.5 ± 3.6	49.5 ± 2.4
30	42.3 ± 2.7	52.8 ± 2.2	47.7 ± 2.2
60	44.3 ± 1.6	48.7 ± 2.7	47.6 ± 2.0
90	40.9 ± 1.2	47.6 ± 0.6	50.6 ± 2.2[c]
120	40.4 ± 0.9	49.3 ± 2.9	50.1 ± 3.2[c]
150	38.9 ± 1.8	46.9 ± 1.8	46.3 ± 3.5
180	40.7 ± 4.4	47.4 ± 3.0	44.2 ± 5.1

[a] Values obtained 5 min. prior to drug administration. [b] Results expressed as maximum percentage inhibition of potentials (mean ± *SE*) for four experiments. [c] Statistically significant ($p = 0.05$) difference from control values.

et al. (9) after intravenous infusion of imipramine at a rate of 80 mcg./kg./min.

Slow (over a 90-sec. period) arterial injection of 250 mcg. or less of DMI had no detectable effect on ganglionic transmission during the 3-hr. experimental period. A dose of epinephrine which depressed the postganglionic potential 49.2% (mean, $N = 4$) before administration of 250 mcg. of DMI, produced a 57.4% reduction 30 min. after DMI, and a 60.9% decrease (maximum effect) 150 min. after DMI administration (Table III). Similar, but less persistent intensification of the action of epinephrine was observed after injection of 125 mcg. of DMI. In this series, epinephrine diminished the postganglionic potential 46.8% (mean, $N = 4$) before DMI, and 56.7% (maximum effect) 30 min. after administration of DMI (Table III).

DISCUSSION

It might be assumed that a functioning adrenergic inhibitory system at ganglionic sites would be associated with pathways for catecholamine inactivation within the ganglia, and that chemical interference with these pathways would result in potentiation of the ganglionic depressant activity of epinephrine.

Previous studies demonstrated sympathetic ganglionic transmission to be blocked (7–9) or unaffected (10, 11) by MAO inhibitors. However, in view of the apparent limited participation of oxidative deamination in terminating the activity of either locally released or circulating catecholamines (12), it is doubtful whether MAO inhibition results in extraneuronal levels of adrenergic amines adequate to depress ganglionic transmission.

Throughout a wide dosage range (3.12–800 mcg.) the effect of close arterial injection of tropolone was negligible on epinephrine-induced depression of transmission in the perfused superior cervical ganglion of the cat. With the exception of a slight enhancement ($p = 0.05$) at the 25-mcg. dose level, tropolone produced no increase in the extent or duration of the ganglionic inhibitory effect of epinephrine. The results of this study suggest that COMT is not involved significantly in the termination of catecholamine activity in sympathetic ganglia.

The process of adrenergic neuronal uptake of catecholamines is considered of major importance in the inactivation of norepinephrine and epinephrine (13, 17, 18). The phase of uptake involving the transfer of catecholamines from extraneuronal sites, through the cell membrane, to intraneuronal storage areas is blocked by various pharmacologic agents, including DMI (19). Imipramine and, to a greater extent, DMI have been reported (20) to enhance the response of the nictitating membrane to preganglionic electrical stimulation and to exogenous norepinephrine and epinephrine.

Table II—Effect of Close Arterial Injection of Tropolone on Epinephrine-Induced Inhibition of Postganglionic Potentials

Min.	Control	Tropolone, mcg. 200	400	800
−5[a]	58.8 ± 0.7[b]	58.1 ± 4.0	53.0 ± 3.1	59.1 ± 5.3
5	58.5 ± 2.2	58.5 ± 4.1	58.5 ± 3.9	47.2 ± 2.3
15	60.9 ± 2.5	55.1 ± 6.1	55.4 ± 4.3	47.0 ± 1.8
30	60.2 ± 4.8	46.9 ± 2.5	47.8 ± 2.4	50.1 ± 4.5
60	53.7 ± 1.9	48.1 ± 4.2	47.5 ± 2.4	41.3 ± 4.0
90	51.5 ± 4.1	46.6 ± 4.1	49.8 ± 2.2	41.8 ± 1.3
120	53.2 ± 6.3	45.4 ± 7.0	42.7 ± 3.9	45.3 ± 5.7
150	46.3 ± 4.8	56.2 ± 3.2	38.7 ± 5.1	36.0 ± 10.4
180	45.9 ± 8.0	51.7 ± 2.3	32.0 ± 12.1	36.2 ± 12.1

[a] Values obtained 5 min. prior to drug administration. [b] Results expressed as maximum percentage inhibition of potentials (mean ± *SE*) for four experiments.

Table III—Effect of Close Arterial Injection of Desmethylimipramine on Epinephrine-Induced Inhibition of Postganglionic Potentials

Min.	Control	Desmethylimipramine, mcg. 125	Desmethylimipramine, mcg. 250
−5[a]	48.0 ± 1.5[b]	46.8 ± 2.1	49.2 ± 2.9
5	46.5 ± 2.2	54.4 ± 4.2	47.7 ± 5.2
15	44.9 ± 2.1	56.3 ± 3.2	54.2 ± 4.2
30	42.3 ± 2.7	56.7 ± 5.1[c]	57.4 ± 4.2[c]
60	44.3 ± 1.6	56.1 ± 4.6[c]	57.1 ± 3.2[c]
90	40.9 ± 1.2	54.6 ± 4.2[c]	58.6 ± 3.5[c]
120	40.4 ± 0.9	50.7 ± 3.9	59.7 ± 4.0[c]
150	38.9 ± 1.8	53.3 ± 4.5[c]	60.9 ± 2.1[c]
180	40.7 ± 4.4	53.2 ± 3.8	57.9 ± 4.7[c]

[a] Values obtained 5 min. prior to drug administration. [b] Results expressed as maximum percentage inhibition of potentials (mean ± *SE*) for four experiments. [c] Statistically significant ($p = 0.05$) difference from control values.

The results of this investigation confirm the ability of DMI to potentiate various biologic effects of exogenous epinephrine. Close arterial injection of 125 and 250 mcg. of DMI resulted in significant increases in epinephrine-induced suppression of transmission in the superior cervical ganglion. In this regard, Cairncross *et al.* (21) found that nortriptyline potentiated the ganglionic inhibitory effect of norepinephrine, although they were unable to demonstrate a facilitation of ganglionic depression induced by epinephrine.

It is suggested that potentiation by DMI of the inhibitory effect of epinephrine on transmission is attributable to reduction in catecholamine uptake within the ganglion. Pharmacologic evidence for the existence of an uptake process for terminating catecholamine activity within sympathetic ganglia, and for the occurrence of ganglionic α- and β-adrenergic sites (22), is consistent with the proposal of an adrenergic inhibitory system in sympathetic synaptic transmission.

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Hemolytic Actions of Short-Chain Alkylamine Hydrochlorides

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Abstract □ The hemolytic actions of a number of short-chain alkylamine hydrochlorides were studied. Primary salts, produced complete hemolysis but secondary, tertiary, and quaternary salts produced incomplete hemolysis. This was attributed to the increased bulkiness and lipophilicity of the successively alkyl-substituted amine cations. In each group of the salts, the hemolytic activity increased with increasing alkyl chain length. The importance of the interaction of the alkylamine cations with phospholipid in the erythrocyte membrane was emphasized on the basis of the amount of lipids released by the cations from erythrocytes.

Keyphrases □ Alkylamine hydrochlorides—*in vitro* hemolytic action □ Hemolytic effect—alkyl chain length □ Erythrocyte aggregation—alkylamine hydrochlorides

A previous paper (1) has reported a remarkable difference in hemolytic activity between short-chain anionic and cationic surface-active electrolytes. Thus, sodium alkyl sulfates and sodium carboxylates with shorter hydrophobic chain than hexyl radical are hemolytically inactive whereas the corresponding members of alkylamine hydrochlorides and alkyl pyridinium iodides are capable of causing hemolysis. Furthermore, the release of a considerable amount of phospholipids by the surface-active cations were detected by means of TLC prior to lysis (1, 2).

These findings have prompted the study of the hemolytic actions of a number of short-chain alkylamine hydrochlorides in relation to their molecular structure.

EXPERIMENTAL

Preparation of Short-Chain Alkylamine Hydrochlorides—The amine salts were prepared by passing dry hydrogen chloride through benzene solutions of the amines. The precipitated salts were collected and purified by recrystallization from ethanol.

Hemolysis of Dog Erythrocytes—The method of preparing the erythrocyte suspension from dog blood was the same as that used in the previous work (1) except that aqueous 0.9% NaCl solution was employed as the washing liquid and the suspending medium of erythrocytes instead of the isotonic phosphate buffer solution.

The percent hemolysis was estimated, as in the previous work (1), by determining spectrophotometrically the amount of released hemoglobin in the supernatant liquid by the amine hydrochlorides after centrifuging the unhemolyzed cells. The concentration of erythrocytes was 2.5% v/v. Some of the amine salts used in this work caused the aggregation of erythrocytes at low concentrations, and the aggregates formed were observed under a microscope and photographed.

Determination of the Amount of Lipids Released—The procedures of determining the amount of lipids released from the erythrocytes were identical with those adopted in the previous work (2).

RESULTS AND DISCUSSION

With the secondary, tertiary, and quaternary salts, complete hemolysis was not observed presumably due to the limited solubility of these salts and a strong interaction with the released hemoglobin. Figure 1 shows a typical hemolysis curve for mono-, di-, and triethylamine hydrochlorides.

In Table I are listed the hemolytic concentrations of the amine salts, determined from the hemolysis curves, at three different degrees of hemolysis.

The hemolytic activity increases with increasing alkyl chain length in each group of the primary, secondary, and tertiary salts. The same is true of the quaternary salts though the results are not given in the table. Thus, the hemolytic concentrations required of tetramethyl, tetraethyl, and tetrabutyl ammonium chlorides to produce 10% lysis were 1.5, 1.0, and 0.5 moles/l., respectively. These results would come from the increasing surface activity of the salts with alkyl chain length.

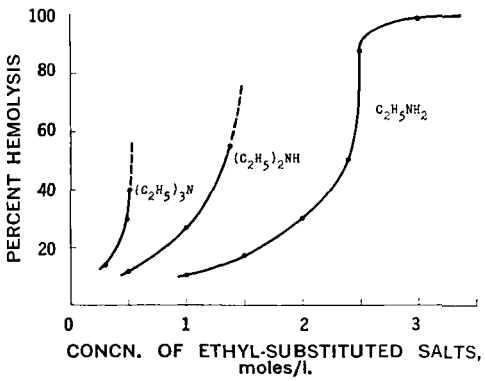

Figure 1—*Hemolysis curves for mono-, di-, and triethylamine hydrochlorides.*