SCREENING OF SOME BASIC AMIDES FOR LOCAL ANAESTHETIC AND NEUROMUSCULAR BLOCKING ACTIVITY

HARIT R. DERASARI AND MAHESH N. PATEL

Department of Pharmacology, L.M. College of Pharmacy, Ahmedabad-9.

Summary: Of the twenty six basic amides synthesised, 12 were derivatives of N-substituted phenoxypropyl nucleus (group I), 8 were derivatives of N-substituted phenoxyethyl nucleus (gruop II) and 6 were derivatives of X-secondary amino- N-phenylethyl nucleus (group III).

All the 26 compounds were first screened for their local anaesthetic activity on rabbit cornea. Only 12 compounds which exhibited dose-dependant surface anaesthetic activity were investigated for their neuromuscular blocking activity. These were Compounds C,D,F,G,H,J, and L from group I; Compound S from group II and compounds U,W,X, and Y from group III. These 12 compounds produced dose-dependant block of responses of isolated phrenic nerve-diaphragm preparation of rat to indirect stimulation. The block was not antagonised by neostigmine or potassium chloride. D-tubocurarine and calcium chloride potentiated the block.

The compounds antagonised noncompetitively the stimulant action of acetylcholine on the isolated rectus abdominis muscle of frog and blocked responses of chick—biventer cervices muscle preparation to indirect stimulation. The block was not affected by neostigmine. It is concluded that the compounds may have a presynaptic blocking action.

Key words: basic amides local anaesthetic activity neuromuscular blocking activity presynaptic action

INTRODUCTION

Twenty six basic amides, chemically different from lignocaine which is an aminoacyl amide, were recently synthesised by Trivedi *et al.* (6,7,8). These compounds were first screened for their local anaesthetic activity and of them twelve compounds were found to be active as surface anaesthetic agents, in concentrations upto 1%. The active compounds were further studied for their effect on the neuromuscular junction.

As can be seen from Fig 1 compounds 1-12 are derivatives of N-substituted phenoxypropyl nucleus, compounds 13-20 are derivatives of N-substituted phenoxyethyl nucleus and compounds 21-26 are derivatives of X-secondary amino N-phenylethyl nucleus. The various substituents and code names are detailed in Table I.

MATERIALS AND METHODS

Local anaesthesia:

The agents were tested on rabbit's cornea for surface anaesthesia in concentrations of 0.02—1% by the method of Chance and Lobstein (3).

TABLE I: Substituents used for the compounds whose nuclei are shown in Fig. I. The code names of the l active compounds are given.

Sl. No.	*X*	*Y*	*Code name*	*Sl. No.*	*X*	*Q*	*Code name*
1.	O-CH_3	Morpholine	—	14	O-CH_3	Morpholine	—
2.	p-CH_3	Morpholine	—	15	O-CH_3	Piperidine	—
3.	p-CH_3	Piperidine	C	16	O-Cl	Morpholine	—
4.	p-CH_3	N-Et_2	D	17	O-Cl	Piperidine	—
5.	O-Cl	Morpholine	—	18	p-CH_3	Morpholine	—
6.	O-Cl	N-Et_2	F	19	p-CH_3	Piperidine	S
7.	p-Cl	Morpholine	G	20	p-Cl	Moropholine	—
8.	p-Cl	N-Et_2	H	21	3:4, dimethyl	Peperidine	U
9.	2:4. Cl_2	Morpholine	—	22	3:4, dimethyl	Morpholine	—
10.	2:4 Cl_2	N-Et_2	J	23	2:4, dimethyl	Diethylamine	W
11.	2:5. Cl_2	Morpholine	—	24	2:4, dimethyl	Piperidine	X
12.	2:5, Me_2	Morpholine	L	25	2:5, dimethyl	Piperdine	Y
13.	H	Piperidine	—	26	2:5, dimethyl	Morpholine	—

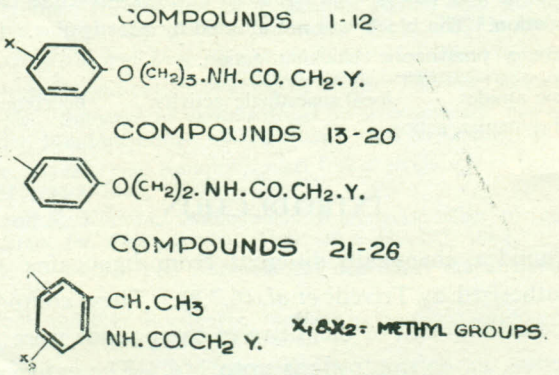

Fig. 1

Neuromuscular blocking action:

Phrenic nerve diaphragm preparation of rat: Hemi-diaphragm of rat with phrenic nerve attached was dissected out and suspended in a bath of 50 *ml* capacity containing oxygenated Tyrode solution with double the amount of dextrose as suggested by Burn (2). Contractions of the diaphragm were induced by square wave pulses indirectly by stimulating the phrenic nerve and directly by stimulating the muscle. The strength of the current was 0.2 to 2 *ma* and the duration of pulse was 0.2 *ma* for both types of stimulation. The stimuli were applied for not more than 12 times per min.

All the compounds were studied in three graded concentrations. The potencies of the compounds were compared with that of d-tubocurarine. The effects of neostigmine, potassium chloride, d-tubocurarine, calcium chloride, choline chloride and tetanic stimuli (50/sec.) of the same strength and pulse width as mentioned obove were observed on the partial blockade produced by the compounds.

Rectus abdominis muscle of frog: The method was as described by Burn (1). Cumulative dose response curves were obtained with acetylcholine and their modification by the compounds was studied.

Isolated chick biventer cervices nerve muscle preparation: Male chickens weighing 200-250 *g*, were killed by head blow and biventer cervices nerve muscle preparations were set up by the method of Ginsborg and Warriner (4).

Gastrocnemius sciatic nerve preparation of Cat : Male cats weighing 2 to 2.5 *kg*, were used and experiments were set up as described by Burn (2).

RESULTS

Surface anaesthetic activity :

Twelve compounds showed surface anaesthetic activity which was dose dependent. The median effective dose was calculated for each compound (Table II). The surface anaesthetic

TABLE II: Median effective doses* (MED) of lignocaine, cocaine and 12 active agents for surface anaesthetic activity and for block of responses to indirect stimulation of phrenic nerve diaphragm preparation.

Compound	*Surface anaesthetic activity*	*Phrenic nerve diaphragm*
	MED (*g*/100 *ml*)	*MED* (*mg*/100 *ml.*)
Lignocaine	0.23	1.318
Cocaine	0.25	1.92
C	0.06	0.87
D	0.22	1.10
F	0.32	1.45
G	0.50	2.40
H	0.34	1.50
J	0.15	1.10
L	0.6	2.7
S	0.33	2.3
U	0.18	1.26
W	0.33	1.74
X	0.25	1.26
Y	0.54	2.5

* Means of three experimental values.

activity was compared with that of cocaine and lignocaine. Out of the twelve active compounds, compounds C,J,D, and U were found to be more active than cocaine and lignocaine, while compounds, F, H, S, W, G, Y and L (arranged in descending order of potency) were found to be less active than cocaine and lignocaine. Compound X was equipotent to cocaine, but less potent than lignocaine.

Neuromuscular blocking activity :

Phrenic nerve diaphragm of rat: Graded doses of the twelve compounds and those of cocaine and lignocaine were added to the bath in concentrations of 6 to 64 $\mu g/ml$.

With all compounds and the standard drugs, cocaine and lignocaine, there was a reduction in the height of diaphragmatic contraction by indirect stimulation. The reduction was dose dependent (Fig. 2). Responses to direct stimulation were not affected. The median

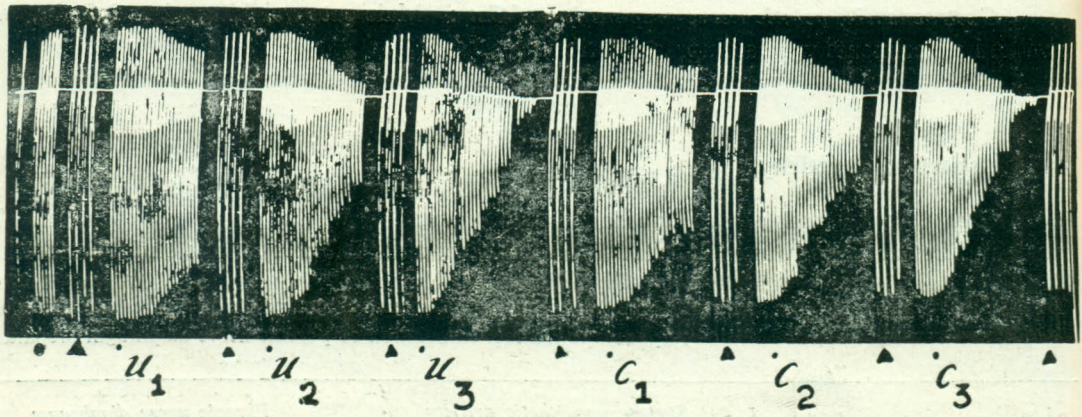

Fig. 2: Responses of phrenic nerve diaphragm to indirect stimulation (at dots; 0.2-2 ma for 2 ms/5 sec) and direct stimulation (at triangles; 2 ms/5 sec.) U_1 U_2 and U_3 indicate 8 $\mu g/ml$, 16 μg /ml and 32 $\mu g/ml$ respectively of compound U, and C_1, C_2 and C_3 indicate 6 $\mu g/ml$, 12 $\mu g/ml$ and 24 $\mu g/ml$ respectively of compound C.

effective dose of each compound was calculated. Compounds C,J,D,U and X were more potent than cocaine and lignocaine. Compounds F, H, and W were more potent than cocaine but less potent than lignocaine while compounds S,G,Y and L were less potent than lignocaine and cocaine. The times of recovery to the initial height of contraction after several washes ranged from 12 to 20 min with different compounds.

Neostigmine (10 $\mu g/ml$) and potassium chloride (200 $\mu g/ml$) were able to counteract the partial block produced by cocaine and lignocaine (Fig. 3 and 4) while they were ineffective in counteracting the partial block produced by all the compounds under study (Fig. 5). d-tubocurarine (2 $\mu g/ml$) and calcium chloride (100 $\mu g/ml$) increased the blockade produced by all the compounds as well as that by cocaine and lignocaine. Choline chloride (5 $\mu g/ml$) had no effect on the block produced by the compunds under study, as well as that produced by

cocaine and lignocaine. However, the diaphragmatic muscle responded to tetanic stimulation after the block produced by all the twelve compounds (Fig. 3, 4 and 5).

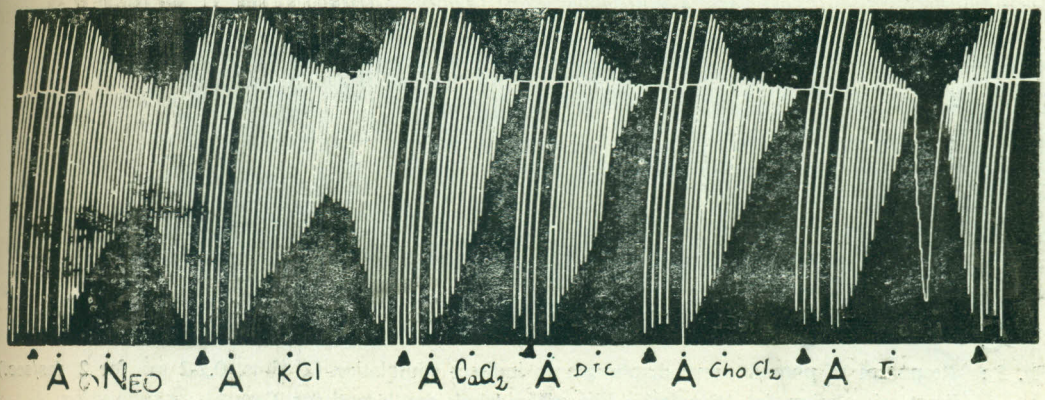

Fig. 3: Responses of phrenic nerve diaphragm to indirect stimulation (at dots 0.2-2 ma for 2 m/5 sec.) and direct stimulation (at triangles). A indicates cocaine (15 $\mu g/ml$); Neo indicates neostigmine (10 $\mu g/ml$); KCl indicates potassium chloride (200 $\mu g/ml$), $CaCl_2$ indicates calcium chloride (200 $\mu g/ml$), DTC indicates d-tubocurarine (2 $\mu g/ml$, Cho.Cl_2 indicates choline chloride (5 $\mu g/ml$) and Ti indicates tetanic stimuli 50/sec.

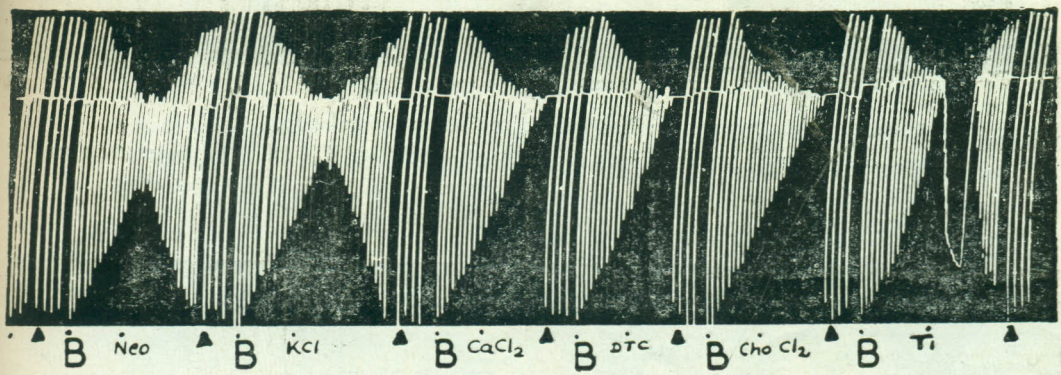

Fig. 4: Responses of phrenic nerve diaphragm to indirect stimulation (at dots, 0.2-2 ma for 2 ms/5 sec.) and direct stimulation (at triangles). B indicates lignocaine (25 μg). Details of Neo, KCl, $CaCl_2$, DTC Cho.Cl_2 and Ti are the same as in Fig. 3.

Rectus abdominis muscle of frog: The dose response curves of accetylcholine in the presence of these compounds were shifted to the right in a non-parallel fashion but with the standard drugs (cocaine, lignocaine and d-tubocurarine) the shift was parallel.

Chick-biventer cervicis nerve muscle preparation: In three experiments each, all the compounds including cocaine and lignocaine produced neuro-muscular blockade. The block produced by cocaine was reversed by neostigmine (Fig. 6 and 7) while that by the other compounds was not.

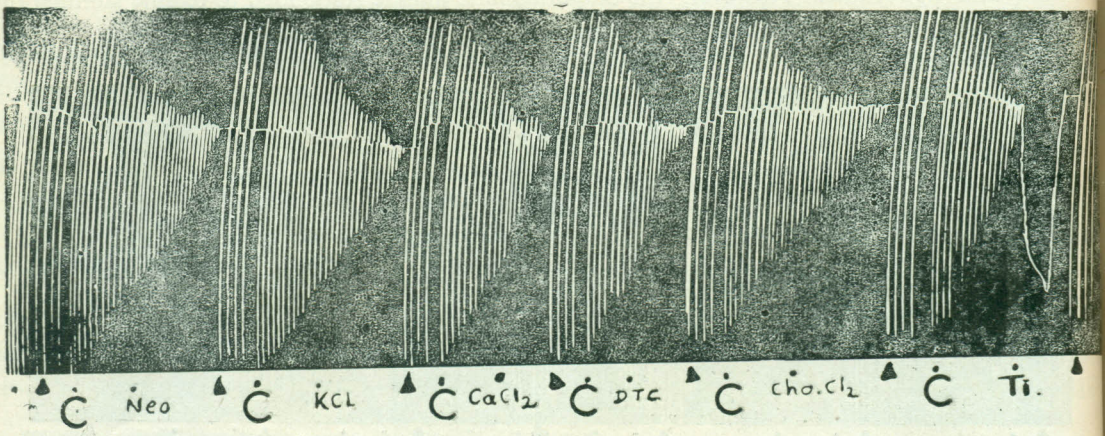

Fig. 5 : Responses of phrenic nerve diaphragm to indirect stimulation (at dots, 0.2-2 ma for 2 ms/sec.) and direct stimulation (at triangles). For other details see legend for Fig. 3.

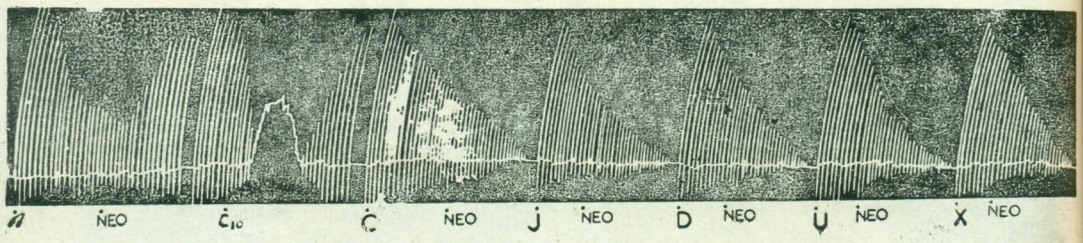

Fig. 6: Responses of chick biventer cervices muscle to indirect stimulation.
A indicates coaine (20 μg) NEO indicates neostigmine (10 μg) C_{10} inidcates decamethonium (10 μg), C indicates compound C (10 μg), J indicates compund J (20 μg), D indicates compound D (20 μg), U indicates compound U (25 μg) and X indicates compound X (25 μg).

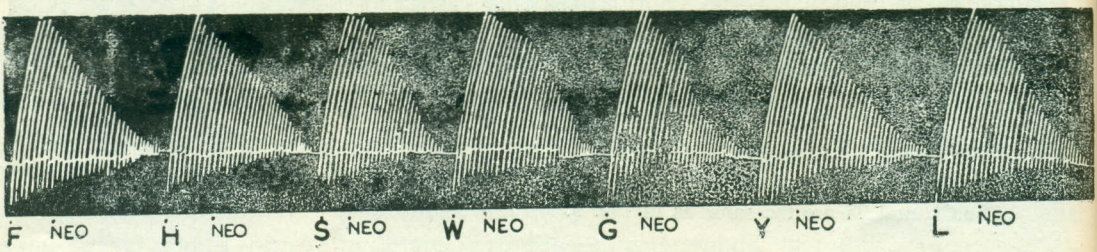

Fig. 7: Responses of chick biventer cervices muscle to indirect stimulation. F and H indicate compounds F and H (30 μg each), NEO indicate neostigmine (10 μg), S and L indicate compound S and L (50 μg each) W, G, and Y indicate compounds W, G and Y (40 μg each.)

DISCUSSION

Twenty six compounds were screened for their local anaesthetic activity. Twelve compounds which were found to be relatively more active were taken up for the detailed study

of neuro-muscular blocking property. The compounds under investigation, chemically resemble pramoxine [4-(3-(p-butoxyphenoxy)propyl)-morpholine) and dimethisoquin [3-(3-butyl-1-(2. dimethyl aminoethoxy)-isoquinoline] which is used clinically for surface anaesthesia.

During the course of study compounds C,D,F,G,H,J and L were found to be active from group 1, compound S was active from group 2 while compounds U,W,X and Y were found to be active from group 3. From the activity profile of the twelve active compounds, it appears that a decrease in intervening aliphatic chain decreases the surface anaesthetic activity considerably. From 12 compounds of group 1 having propyl group as intervening aliphatic chain, seven active compounds were found while from group 2 having ethyl group as intervening aliphatic chain, only one active compound was found. Substitution of phenoxy-propyl group by phenylethyl group as in 6 compounds of group 3, increased the activity and four active compounds were found.

Compound C from group 1 was found to be most active amongst all the twelve compounds containing piperidine ring. Compound S, the only active compound from group 2 also possesses piperidine ring at the position Y. Compounds U, X and Y belonging to group 3 also have piperidine ring. Substitution at position Y with diethylamine group also gave five active compounds which include compounds D, F, and J from group 1 and compound W from group 3. Substitution at position Y with morpholine gave only two active compounds (G and L from group 1) but these compounds were the least active from among the twelve active compounds.

Compounds which have propyl group as intervening aliphatic chain and piperidine substitution at position Y are highly active surface anaesthetic agents (compounds C and U). Substitution with piperidine ring at position Y and phenylethyl group instead of phenoxypropyl, also gave active compounds. Substitution in the intervening aliphatic chain with ethyl and in the Y position with morpholin group, led to decreased activity.

Concentrations of the compounds producing 100% reduction in the height of contraction to indirect stimulation of rat diaphragm, did not affect responses of diaphragm to direct stimulation. Thus it could be concluded that all the drugs affect transmission of impulses at the myoneural junction.

The actions of compounds on the rat phrenic nerve diaphragm and the rectus abdominis muscle of frog were reversible and dose dependent.

Neostigmine and potassium chloride which could counteract the neuromuscular blockade produced by d-tubocurarine, lignocaine and cocaine, were unable to antagonise the partial block produced by any of the compounds under study. This experimental evidence, points to non-involvement of post-synaptic receptor occupation, through which actions of acetylcholine and d-tubocurarine are mediated. However, the compounds may be depolarizing

in nature like decamethonium. Neuromuscular block produced by the compound under investigation was not antagonised by d-tubocurarine. At the same time none of the compounds produced initial depolarisation. This is suggestive of non-involvement of deplorisation mechanism.

Thus neuromuscular blockade produced by all the compounds is neither d-tubocurarine like nor decamethonium like. The drugs having local anaesthetic activity act pre-synaptically, inhibiting the release of acetylcholine. It is quite likely that the compounds studied may have such type of complex interaction at presynaptic level which remained undetected during the course of investigation. However, the interaction between the neuro-muscular blocking action of these compounds and choline chloride suggests that the compounds do not have a hemicholinium like action (5).

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