CHAPTER VI

GENERAL DISCUSSION AND THERAPEUTIC POSSIBILITIES.
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Since the discovery of the first synthetic local anaesthetic procaine by Einhorn (1905), attempts have been made to synthesise newer and better compounds from the point of view effectiveness and toxicity. Lignocaine is a recent addition to the list of synthetic local anaesthetics, which has proved superior in many ways to the existing compounds but not ideal. The present study was undertaken to evaluate the local anaesthetic potency and general pharmacological actions of a series of analogues of lignocaine whose chemical nature and development has been thoroughly discussed in the chapter on chemical consideration in this thesis. These analogues were compared with the older compounds i.e. cocaine, procaine and lignocaine for their local anaesthetic potency.

It is very much evident from the results that some of the tested compounds have been found more potent than the older drugs. Compound F (Table 1 and graph 1) has been found 4 and 2 times more potent as a surface anaesthetic than lignocaine and cocaine respectively. The same compound has been found 4 to 8 times more potent as a block anaesthetic (intradermal wheal in guinea pig) than lignocaine and procaine.
respectively. Similarly compound A has been found 4 times more potent than procaine as a block anaesthetic (Table 3 and graph 3). The rest of the compounds have shown varying degree of anaesthetic potencies, some of which are slightly more or less potent than the reference drugs. The duration of anaesthesia has also varied according to the concentrations and the nature of the compounds. The local anaesthesia produced by all these compounds was reversible and normal sensitivity could be restored in a reasonable period of time (Table 1, 3; Graph 2,4).

Chemically these local anaesthetic compounds could be divided into two groups i.e. the acetamide compounds and the propionamide compounds. The maximum local anaesthetic potency has been shown by one of a propionamide compound F which has a 2:4 dimethyl benzene and \textit{piperidenyl} linkage. The next in the order of the potency is an acetamide compound 'A' having an ortho chloro benzene and a diethyl amino linkage. Number 3 in the series for surface anaesthesia is again an acetamide compound C but has a \textit{piperidenyl} group as in compound F. Next in the series is also an acetamide compound B which has a morpholinyl group linked with 2:4 dimethyl benzene ring. The last in the series are compounds D and E which are both propionamide compounds. Compound D has a diethyl amino group like compound A and a 2:4 dimethyl benzene ring like compound F but none of these groups have lent the potent surface anaesthetic property of these compounds.
It is therefore obvious that none of the different groupings present in compounds are alone responsible for their maximum surface anaesthetic property but the specific combination of these groups together seems to have resulted in a specific local anaesthetic activity.

None of the compounds showed any sign of toxicity such as irritation, smarting, inflammation, necrosis etc. at the site of application of the drugs. Systemic reactions on local application or after injection were also absent.

Toxicity of lignocaine has been determined by Hazard et al. (1951) and Wieling (1952) in different species of animals. In the present study acute toxicity of these compounds has been determined in mice and L.D. 

It was generally observed that those compounds possessing maximum local anaesthetic activity also showed maximum toxicity, except in case of the reference drug lignocaine and compound D. Lignocaine proved more toxic than compound F while compound D which is No. 5 regarding surface anaesthesia, is almost as toxic as compound F.

However, if local anaesthetic activity is taken into consideration, compound F should be considered as
the safest. Scrutinizing the results of surface and intradermal anaesthesia the approximate ratio of potency was 1/2, 1/8, 1/4, 1/12, 1/12, 1, and 1/4, for A, B, C, D, D, F and L respectively in relation to the potency of compound F which was taken as 1 or 100%.

The toxic symptoms as observed in mice were those related to the central nervous system and respiratory system. These were in the nature of exaggerated breathing soon after injection of drug, followed by depression and difficulty in breathing. Sometimes unconsciousness and cheyne-stokes respiration was also observed.

Effect of these compounds was also investigated after their prolonged administration in young albino rats. All the drugs were administered in doses of 6 mg/kg (1% concentration) subcutaneously for a period of one month. Observations were made regarding the weight, tissue injury at the site of injection or any change in behaviour and mortality during this period. It was observed that there was no remarkable change in weight, tissue injury at the site of injection nor any death during the period of observation. On completion of one month's period of observation, the animals were killed and some of the internal organs like heart, lungs, kidney suprarenal and spleen were removed for histo-pathological examination. Histo-pathological examination does not reveal any gross or microscopic changes in these organs.
In order to investigate the effect of these local anaesthetics on the cardiovascular system, experiments were done, on arterial blood pressure of dog, peripheral blood, vessels of frog and dog, coronary blood vessels of rabbit, vasomotor centre of dog, autonomic ganglion of dog, myocardium of frog and rabbit and on normal and fibrillating heart of dog.

The effect of arterial blood pressure was studied on both the normal and spinal dogs. These drugs produced fall in arterial blood pressure with all the doses ranging from 1 to 5 mg/kg in normal and spinal dogs. The hypotensive effect was proportionate to the doses administered. The maximum fall in blood pressure in both the cases was observed with compounds A, C, D & F - while it was least with compounds B & E. (Fig. 1 to 7, 23 24 & 26.)

The hypotensive activity remained unaltered even after administration of full doses of atropine and antihistaminic drug Mepyramine Maleate (Fig. 1 to 7, &28 to 14). The action of adrenaline, acetylcholine and histamine also remained unchanged after administration of these compounds (Fig. 15 to 21.)

The fall in arterial blood pressure was more marked against ephedrine hydrochloride (0.5 mg / kg) induced hypertension (Fig. 22 to 26.)

There are contradictory reports by various workers. (Anrep 1880, Bran 1941, Hazard 1946, Edrmonds et al 1949, Formmel et al. 1950, Haggart wood 1951, Hazard 1951

Results obtained with these compounds reveal that the hypotension is not due to cholinergic or histamine like action of drugs nor there is any relationship of the hypotension with the action of the chemical mediators of the body.

The hypotensive effect in the spinal dog is nearly the same as in normal animal which indicates that the effect is not mediated through the central nervous system. The effect is however, more marked against the induced hypertension with ephedrine (Fig. 23, 24, 26). This might be of importance in clinical practice involving hypertension.

In order to investigate the peripheral sites of hypotensive action, these compounds were studied on perfused blood vessels of frog and hind limb vessels of dog. All the compounds given in graded doses produced vasodilatation which was roughly proportionate to the dose administered. Compound F and A were found to be most potent as vasodilatory agents. The least potent was compound B and E (Table No. 7, graph 5 & 6). These results correspond well with those observed on the blood pressure since the vasodilatory and the hypotensive
properties are approximately parallel. Hence the direct effect on the smooth muscles of peripheral blood vessels may be considered as one of the factors responsible for causing hypotension.

Effect of these compounds was also studied on coronary blood vessels of rabbit. The results are varied. Compounds D & F have shown coronary dilatation while compound C has shown constriction with all the doses administered. Compounds B, E & L have very little effect on total coronary output, while compound A has shown an initial marked dilatation followed by constriction with all the doses administered (Fig. 55 to 61, Table 8). If the results could be applied to human beings, use of the compound C should be withheld in patients of coronary thrombosis but the compounds D & F may be used with advantage since they are potent coronary dilators.

The drugs depressing vasomotor centre could also effect a fall in blood pressure, therefore the effect of these compounds was investigated on the vasomotor centre of dogs. According to Mary's law a fall in blood pressure in the carotid sinus reflexly stimulates the vasomotor centre resulting in a rise of blood pressure. The fall in blood pressure in the carotid sinus could be produced by clamping both the common carotid arteries below their bifurcation. If the drug depresses the vasomotor centre, the rise in blood pressure, after clamping the common carotid arteries, will not be the same after the drug as normally. It was observed by
Anrep (1880) and Heymans et al. (1933) that procaine and cocaine have some effect on carotid sinus reflex. Hence these compounds were studied with a view to investigate their action on vasomotor centre and to determine whether the hypotensive action is in any way connected with it. It is evident from the results (Fig. 27 to 33) that these drugs have not altered the rise of blood pressure on clamping both the common carotid arteries thus eliminating the vasomotor centre and the carotid sinus as the possible site of action.

In order to investigate further the mechanism of the fall in blood pressure with these compounds, experiments were conducted to eliminate the possibilities of their action on the autonomic ganglion. The effect of these compounds was therefore determined on nictitating membrane of dogs. Some workers (Hazard, 1949; MacGregor, 1939; and Harvey, 1934) have observed that the older local anaesthetics cocaine and procaine have some effect on autonomic ganglion. This has been confirmed in the present experiments on the nictitating membrane of dog (Fig. 34 to 47). Compound E & F were found to be the most potent in reducing the height of contraction of nictitating membrane after pre-ganglionic stimulation (Fig. 34 to 40, Table 9) while compounds A and L were most potent in reducing contraction after post-ganglionic stimulation. (Fig. 35 to 47, Table 9 graph 7). Thus the hypotensive action may be partly
mediated through the effect on sympathetic autonomic ganglion and sympathetic nerve endings.

The effect of these drugs was studied on the isolated perfused hearts of frog and rabbit. On the frog's heart the effect was found to be variable. Compounds A, D, F and L depressed the heart with all the doses administered (Fig. 48, 51, 53) while compounds B and E depressed the heart initially followed by stimulation (Fig. 49, 54). Compound C has shown a typical digitalis like action i.e. decrease in heart rate accompanied with marked increase in force of contraction (Fig. 50). The effect on heart remained unaltered after atropinisation (Fig. 48 to 54). On rabbit's heart, all the compounds have shown depressant effect with all the doses ranging from 10^{-3} to 1 mg. This effect was roughly proportionate to the doses administered. The depressant effect remained almost the same after atropinisation (Fig. 55 to 61). Compound A was found to be the most depressant while compound B as least of all the compounds tested in the present series. Compound C has shown well marked cardiac tonic properties on the heart of frog. The effect is very much like that of digitalis. However, the same results could not be seen with the mammalian heart. As such the possibility of its use as a cardiac tonic in human patient can not be anticipated. The depressant effect on the hearts of frog and rabbit with the rest of the compounds
seem to be the result of direct action on the myocardium and may partly account for the hypotensive action of these drugs.

The effect of these compounds was also investigated on the isolated auricle of rabbit, in doses ranging from 0.2 to 40 \( \gamma/\) cc. Some of the compounds i.e. B, C, D & F were found to be stimulant to auricular contractions in smaller doses but depressant in higher doses (Fig. 63, 64, 65, 67) while compounds A, E & L depressed it in all the doses administered (Fig. 62, 66 68). The effect on auricles with compounds B, C, D & F resembles that of procaine as reported by Roth (1917) Macgregor (1939). Procaine and its derivative procainamide have been used extensively in clinical cases of arrhythmias of heart (Mautz, 1936; Wiggers et al., 1940; Burstein, 1940; Hirschfelder, 1942; Dawes, 1946 Wedd et al., 1951). It was therefore thought worthwhile to investigate these compounds from this point of view.

Fibrillation was produced in dogs by means of the method described fully in chapter I, section F. E.C.G. was recorded throughout. None of the compounds tested produced any change on normal E.C.G. pattern except reducing the rate (Fig. 69 to 77 & Table No.11). The effect of these compounds on the fibrillating heart was immediate and significant which was seen by the naked eye as well recorded by E.C.G. The results are indicated in Fig. 78, 79 and Table No.12.
The antifibrillatory effect of these compounds in experimental animals lasted for at least 30 minutes and during this period any attempt to induce fibrillation again was not successful. Maximum duration of antifibrillatory effect was seen with compound C which was approximately 90 minutes. The results when compared with quinidine showed that the antifibrillatory effect with these compounds was more immediate and also marked persistant. The antiarrhythmic property exhibited by these compounds surely deserves clinical trials in arrhythmias of the human heart.

Effect of these compounds was also studied on the respiration of anaesthetised dogs. Initially all the compounds increased the rate and amplitude of respiratory movements which was followed by slight diminution in the amplitude but marked decrease in the rate of respiratory movements (Fig. 80 to 86). The initial stimulation of respiratory movements seems to be mostly a reflex response to the depressor action on the blood pressor, while the secondary depression of respiration may be on account of depression of respiratory centre.

The study of these compounds on the smooth muscles was done on isolated intestine of rabbit and guineapig, tracheal chain preparation and perfused lungs of guinea-pig, carotid arterial strips of dog and isolated uteri.
of guineapig and rat.

The effect was studied on normal movements of isolated intestine and against the spasm induced with various spasmogens. Graded doses of local anaesthetics ranging from 1 to 20γ/ cc were administered in order to study their effect on normal intestinal movements of rabbits. An inhibitory effect was seen with all the drugs which was directly proportional to the doses administered. However, the degree of relaxation varied from drug to drug (Fig. 87). The effect of these compounds in doses ranging from 1 to 200γ/ cc was studied against the spasm induced by acetylcholine (1 to 4γ/ cc) and Barium chloride (40 to 80γ/ cc). A spasmolytic action was observed in both the cases which was directly related to the doses administered. The approximate equipotent doses of these compounds against acetylcholine and barium chloride induced spasm were 20, 80, 10, 200, 60, 6 and 80γ/ cc of A, B, C, D, E, F and L and 20, 80, 10, 20, 60, 4 and 60γ/ cc of A, B, C, D, E, F & L respectively. It is evident from these results that compound F is most potent as a spasmolytic agent against acetylcholine and barium chloride induced spasm. On scrutiny of these results, it is also evident that all the compounds have approximately similar actions against both the spasmogens except compound C and D which have shown specific antagonizing action against acetylcholine and barium chloride induced
spasm respectively (Fig. 88 to 101, Table 13, 14, Graph 8, 9). The effect of local anaesthetics on isolated guineapig's intestine was studied in graded doses ranging from 1 to 400 mg/ cc against the spasm induced by histamine (0.25 to 0.5 mg/ cc). The spasmolytic action was observed with all the compounds and the effect was proportionate to the doses administered. The effect however, varied with different compounds. The approximate equipotent doses of the compounds A, B, C, D, E, F and L were 4, 200, 50, 200 400, 3 and 400 mg/ cc respectively. Here again compound F was found to be the most effective drug which is also slightly more potent than the antihistaminic drug Mepyramine maleate in this respect (Fig. 102 to 108, Table 15, graph 10).

It is interesting to recall that the compounds which has shown maximum local anaesthetic activity has also shown the maximum spasmolytic action which is true against all type of spasmogens such as acetylcholine, histamine and barium chloride.

The spasmolytic action of these compounds was also seen on tracheal chain and perfused lung preparations of guineapig against histamine induced spasm. In both the sets of experiments the antispasmodic effect was roughly proportional to the doses administered. The antispasmodic potency of the local anaesthetics however, varied from drug to drug. The equipotent doses of the
compounds A, B, C, D, E, F and L on the tracheal chain preparation was found to be 2,300, 100, 100, 1.5 and 300V/ cc respectively. Compound F was found to be the most potent in both the (Fig. 110 to 116, Table No. 16) experiments. It is obvious from these experiments that the local anaesthetic compounds studied, have marked bronchodilatory actions, a property shown by some other local anaesthetics also to a varying degree as reported by Sharma (1960). Slight relaxation was observed with all the compounds in higher doses on the carotid arterial strip preparation of dog. Compound F has again shown the maximum relaxation tendency as compared to other compounds (Fig. 117). From the above results it is evident that these compounds have antispasmodic action against all types of spasmogeners and different tissues of varied origin. Many workers (Goodman & Gilman (1955), Corhn et al. et al (1944), SKera et al. (1960), Hazard et al. (1943), Halpern (1945), Sinha (1953), Arora et al. (1956) and Sharma et al. (1960) have tried to demonstrate relationship between local anaesthetic activity and spasmolytic and bronchodialtory properties. The present study has also demonstrated the existence of such a relationship with the local anaesthetic compounds, since the most potent local anaesthetics such as compound F and A has also shown the maximum spasmolytic activity.

The effect of these local anaesthetics was
studied on the uteri of guineapig and rat in graded doses ranging from 0.2 to 200 \( \frac{V}{cc} \). The effect varied slightly in the two species. All the compounds exhibited an oxytocic action on the guineapig uteri except compound A and F which stimulated in smaller doses but depressed in higher doses (Fig. 118 to 124). On rat’s uterus all the compounds increased the rhythmicity but the force of contraction was depressed with compounds A, B, C, D & E (Fig. 125 to 131). From the results it is evident that lignocaine (L) has shown a powerful oxytocic action on both the species while compounds B and E have shown marked stimulation on guineapig and rat uterus respectively (Fig.119 & 129). There is no published literature available regarding the action of local anaesthetics particularly of lignocaine on uterus. These compounds seem to produce a direct action on uterine musculature since the effect has been seen on isolated tissues.

Some of the local anaesthetics such as lignocaine and compound B have shown powerful oxytocic action, besides this being the property common to all the compounds tested, it is desirable that caution should be exercised to use these compounds in pregnant females in clinical practice of course, for purposes other than local anaesthesia. However, oxytocic properties of these compounds may usefully be employed in therapeutics wherever such an action is desired.
In the present study the effect of these local anaesthetics was studied on frog's rectus abdominis muscle, rat diaphragm and dog sciatic nerve gastrocnemious preparation. The effect was similar in all the species except compounds A, C and E which showed variable effect. On frog's rectus abdominis muscle preparation compound C and E have shown potentiation of acetylcholine induced contractions with all the doses administered. Compounds A and B have potentiated in smaller doses but inhibited in higher doses while compound D and F have inhibited the contractions with all the doses administered (Fig 132 to 138, Table 18). The recovery of the muscle was complete after washing out the bath within a period of 15 to 45 minutes with all the compounds.

On rat diaphragm preparation, compounds A, B and C potentiated the height of contractions in smaller doses but reduced it in higher doses. The rest of the compounds inhibited the contractions which was roughly proportionate to the doses administered. Neostigmine in doses of 10 Y/cc was partially effective in counteracting the inhibition of contraction caused by compound C, D & E but was ineffective against block produced by A, B, F & L. The muscle recovered completely after washing out the drug, though the time of recovery varied from drug to drug and in different doses. The muscle however, responded maximally to direct excitation even when it was
unresponsive to indirect stimulation after complete blockade with the drugs (Fig 139 to 145, Table 19).

On dog's sciatic gastrocnemious preparation all the compounds produced inhibition of contraction with all the doses administered except compounds A and E which potentiated the height of contractions slightly with smaller doses and inhibited with higher doses. Neostigmine (0.1 mg/kg) antagonised the partial neuromuscular block produced by all the compounds except compound E. But in the presence of complete neuromuscular blockade with these compounds, neostigmine was ineffective. The responses to direct excitation of the gastrocnemious muscle remained unaltered even when the muscle failed to respond to indirect stimulation after administration of the drugs (Fig.146 to 152, Table 20). From the above results it is clear that compound F is the most potent as a neuromuscular blocking agent which is also the most potent local anaesthetic. Similarly if all the compounds are compared for their local anaesthetic potency and neuromuscular blocking properties, it could be seen that the two properties run approximately parallel. There is a general agreement that local anaesthetics weaken the neuromuscular transmission in mammals and frogs (MacGregor, 1939, Harvey 1938). According to Krantz & Carr (1961), local anaesthetics prevent passage of impulses at myoneural junction. In the present experiments however, compounds
A, E, B and C have stimulated in smaller doses and inhibited contractions in higher doses. It is therefore possible that some of the compounds might be acting by persistent depolarisation while the others by competitive blockade like curare.

In the present study the effect of these local anaesthetic compounds was also studied on the C.N.S. by assessing its effectiveness against artificially induced convulsions such as maximum electroshock seizure test and maximal metrazol test. All the compounds were administered intraperitoneally in doses ranging from 50 to 100 mg/kg. Though none of the compounds could prevent completely the animal from tonic extensor component of the electrically induced convulsions, but, all the compounds have decreased the duration of extensor component as compared to controlled animals. Compound B was found to be the most potent in this respect (Table 21). Similarly all the compounds have also lengthened the survival time after Metrazol induced convulsions. But none of the compounds completely protected the animals in doses administered. The death of the animal was however, delayed from 10 to 30 minutes as compared to controlled animals. In this respect compound B was found to be the most potent (Table 22).

Many workers (Gutierrez et al. 1945, Tanka 1955,
Bernhard et al, 1954) have observed some anticonvulsant activity with older local anaesthetics such as procaine and cocaine. The present study has also revealed that some of the compounds have mild anticonvulsant property.

These compounds were also experimented upon rats for determining their analgesic activity. Drugs were administered in doses ranging from 50 to 100 mg/kg intraperitoneally. The results indicate that none of these compounds possess any analgesic property (Table 23).

The effect of these compounds was investigated on the eye of experimental animals and human beings to observe the effect on pupil and conjunctival vessels. Drug solutions were applied in concentrations ranging from 0.1 to 1%. None of the compounds showed any marked action on the eye. However, in higher concentrations (0.1 to 1%) a slight constriction of the pupil was observed with all the compounds. Higher concentrations of compound F some times produced smarting of the eye lasting for about an hour. No such action was seen with other compounds.

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