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# THE ANALYSIS OF THE INHIBITORY EFFECT OF LOCAL ANAESTHETICS AND PROPRANOLOL ON ADRENOMEDULLARY SECRETION EVOKED BY CALCIUM OR ACETYLCHOLINE

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Local anaesthetics are agents which produce their electrophysiological effects in nerve and muscle by interfering with sodium and potassium fluxes (Taylor, 1959; Inoue & Frank, 1962; Goldman & Blaustein, 1966). However, recent evidence indicates that local anaesthetics can also inhibit the movement of calcium ions in muscle and adrenal glands (Feinstein, 1963, 1966; Rubin, Feinstein, Jaanus & Paimre, 1967). The adrenal gland is especially suited for the study of the specific inhibitory actions of local anaesthetics on calcium movement, since calcium, but not sodium or potassium, is essential for the release of catecholamines from the adrenal medulla (Douglas & Rubin, 1961, 1963). The removal of calcium from the perfusion medium abolishes the powerful secretory activity of acetylcholine and excess potassium ($K^+$). Restoration of calcium to the perfusion fluid causes an immediate release of large quantities of catecholamines. This stimulant action of calcium is antagonized by the local anaesthetic, amethocaine (Rubin *et al.*, 1967). Furthermore, amethocaine profoundly inhibits calcium exchangeability in the adrenal gland and can depress both the increased catecholamine output and the enhanced radiocalcium uptake observed during stimulation by acetylcholine (Rubin *et al.*, 1967).

It seemed of interest to study the effects of other local anaesthetic agents on medullary catecholamine release to see if the ability of a given agent to block calcium-evoked release could be correlated with its local anaesthetic potency as measured in other test systems. In addition, a comparison was made between the efficacy of a given agent to inhibit calcium-evoked release and its efficacy to inhibit acetylcholine-evoked release; one could thus discern whether the depression of acetylcholine-evoked release by local anaesthetics was closely linked to their ability to block calcium movement. Finally, propranolol, a potent $\beta$-adrenoceptive receptor inhibitor, was also examined for its ability to depress calcium-induced catecholamine secretion, since this agent also possesses local anaesthetic activity (Morales-Aguilerá & Vaughan-Williams, 1965), and can interfere with calcium binding to phospholipids *in vitro* (Nayler, 1966).

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## METHODS

### *Perfusion and catecholamine assay procedure*

Anaesthesia was induced in cats (1.5–2.5 kg) with ethyl chloride and ether and then maintained with chloralose (100 mg/kg intravenously). Following tracheal cannulation, the animal was eviscerated and the acutely denervated left adrenal gland was prepared for perfusion *in situ* (Douglas & Rubin, 1961). In one experiment, an adrenal gland, which had been denervated 11 days before the experiment, was perfused as described by Rubin & Jaanus (1966). Perfusion was carried out at room temperature with Locke solution or a modification of the normal Locke solution which contained 10 times normal KCl (56 mM); the NaCl was reduced by an equivalent amount to maintain isotonicity. In some experiments the 2 mM calcium chloride normally present in Locke solution was either omitted or reduced to 0.5 mM, while in other experiments excess calcium (up to 6 times normal) was added. All solutions were equilibrated with 95% $O_2$ and 5% $CO_2$. The perfusate was analysed for catecholamines by a modification (Rubin & Jaanus, 1966) of the photofluorometric technique of Anton & Sayre (1962), and the outputs were expressed as total catecholamines ($\mu$g adrenaline plus noradrenaline base/min).

### *Agents used*

The following agents were generously supplied: propranolol HCl (Inderal; Ayerst); dimethyl aminoethyl p-aminobenzoate (dimethylprocaine; Abbott–1817); cinchocaine HCl (Nupercaine; Ciba). The following drugs were also used: acetylcholine chloride, hexamethonium chloride, and atropine sulphate (Nutritional Biochemical); cocaine HCl and nicotine (Eastman Chemical); amethocaine HCl (Mann); and procaine HCl (Sigma). The concentrations of all agents are given in terms of mole/l.

### *Determination of inhibitory activity*

Since repeated stimulation of the perfused cat adrenal gland causes a progressive decrease in the rate of catecholamine release (Rubin & Jaanus, 1967), the degree of inhibition by a given agent was determined by relating the second evoked output as a percentage of the first. In the absence of inhibitor, the second 2 min stimulation with 0.5 mM calcium was $39.7 \pm 1.76\%$ (n=7) of the first, and with 2 mM calcium $35.8 \pm 1.7\%$ (n=4) of the first. The second response to acetylcholine $6 \times 10^{-6}$M ($10^{-6}$ g/ml.) was $71.6 \pm 2.1\%$ (n=8) of the first, and with nicotine $1.2 \times 10^{-6}$M as the stimulating agent the second response was decreased to about the same extent. Thus, the inhibitory activity of a local anaesthetic was calculated on the basis of the extent to which it depressed the ratio of the second response to that of the first. For example, since the second response to 0.5 mM calcium in the control experiment was $39 \pm 1.76\%$ of the first one, any value below 36.2% (2 standard errors from the mean) was considered to be due to inhibition of catecholamine output by the local anaesthetic. To estimate the $ED_{50}$ for inhibition, linear dose-response curves were obtained by expressing percentage inhibition in probits (Finney, 1964).

## RESULTS

### *The inhibition of calcium-evoked secretion by local anaesthetics*

Amethocaine ($3 \times 10^{-4}$M) inhibited by 90% the large rate of medullary catecholamine release accompanying the reintroduction of calcium (2 mM) to a perfusion medium containing 56 mM KCl (Rubin *et al.*, 1967). In the present experiments, the effect of a number of other local anaesthetics on calcium-evoked secretion was studied. Since 2 mM calcium elicits near maximum rates of catecholamine release in the presence of excess $K^+$, 0.5 mM calcium was mainly employed in this study. This lower concentration of calcium evokes outputs which are only about one-third of those obtained with 2 mM calcium, and thus a given inhibitory effect could be measured more accurately.

The effect of cinchocaine on calcium-evoked secretion was studied first, since it is one of the members of the group of local anaesthetics which is more potent than amethocaine.

Cinchocaine was able to produce a dose-dependent inhibition of the secretory response to calcium (Fig. 1). As with amethocaine, this inhibition could be readily antagonized by increasing the calcium concentration (Fig. 1c). The profound depression produced by high concentrations of cinchocaine could not be reversed by perfusion for 5 min in a cinchocaine-free medium (Fig. 1d). This lack of reversal is apparently due to the long

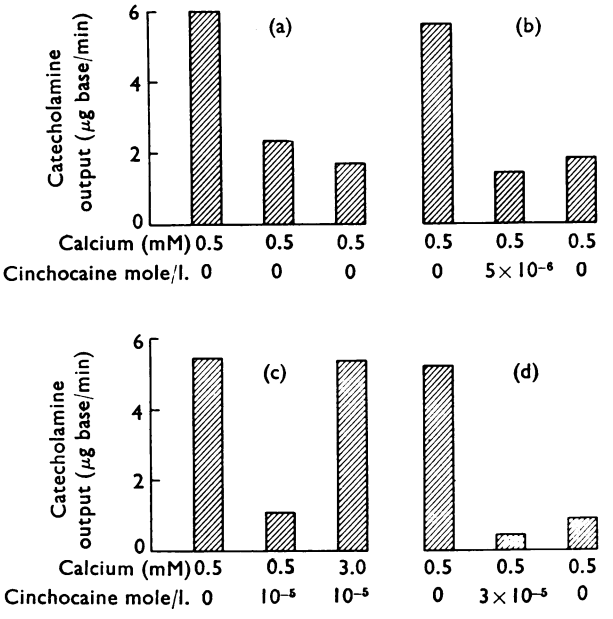


Fig. 1. Inhibition of calcium-evoked secretion by cinchocaine. Adrenal glands were perfused with calcium-free Locke solution containing 56 mM KCl with or without varying concentrations of cinchocaine. At 5 min intervals, calcium (0.5 mM) was added to the perfusion fluid for 2 min. In Fig. 1(c) cinchocaine ($10^{-5}$M) was present for 14 min and 0.5 mM calcium was added during the 5–7th min and 3 mM calcium was added during the 12–14th min of perfusion. The vertical bars represent the catecholamine outputs obtained during the 2 min period when calcium was present both in the presence and absence of cinchocaine.

duration of action of cinchocaine—a property commonly observed in other tissues. Analysis of the dose-response curves of these two local anaesthetics showed that cinchocaine was approximately three to four times more potent than amethocaine in suppressing calcium-evoked secretion (Table 1).

Cocaine and procaine were studied in the same way. Although they were much less potent than either cinchocaine or amethocaine (Table 1), both procaine (Fig. 2b) and cocaine showed a dose-dependent inhibition of the secretion accompanying the reintroduction of calcium, and the inhibition could be readily reversed by excess calcium. For example, procaine ($2 \times 10^{-3}$M) produced a 65% inhibition of secretion evoked by 0.5 mM calcium ; however, catecholamine output was restored to its initial value if in addition to the same concentration of procaine, 3 mM calcium was also present (compare with Fig. 1c).

TABLE 1

A COMPARISON OF THE POTENCY OF LOCAL ANAESTHETICS TO INHIBIT MEDULLARY CATECHOLAMINE RELEASE EVOKED BY THE REINTRODUCTION OF CALCIUM (0·5 mM) FOLLOWING PERFUSION WITH CALCIUM-FREE LOCKE SOLUTION CONTAINING 56 mM KCl

The correlation coefficient of local anaesthetic concentration and degree of inhibition of secretion was derived from dose response curves as shown in Fig. 2. The correlation coefficient of each inhibitor was significant at the $<0{\cdot}05$ level (Student $t$ test). The $ED_{50}$ of each drug (dose required to diminish the secretory response to calcium by 50%) was calculated by the method of least squares. The relative potency ratios were obtained by dividing the $ED_{50}$ of procaine by the $ED_{50}$ of the given local anaesthetic.

| Drug | Correlation coefficient | $ED_{50}$ | Relative ratio (procaine-1) |
|---|---|---|---|
| Cinchocaine | +0·998 | $1{\cdot}3\times10^{-5}$M | 122 |
| Propranolol | +0·973 | $2{\cdot}7\times10^{-5}$M | 57 |
| Amethocaine | +0·938 | $4{\cdot}7\times10^{-5}$M | 33 |
| Cocaine | +0·974 | $1{\cdot}4\times10^{-4}$M | 11 |
| Procaine | +0·979 | $1{\cdot}5\times10^{-3}$M | 1 |

Propranolol was also found to be a very potent inhibitor of calcium-evoked catecholamine release (Fig. 2c). Only cinchocaine proved to be more effective than propranolol in suppressing the increase in catecholamine secretion resulting from the reintroduction of calcium (Table 1). The inhibition by propranolol could be overcome by increasing the calcium concentration. Thus, propranolol ($8\times10^{-5}$M) produced a 90% inhibition of secretion evoked by 0.5 mM calcium. The addition of 3 mM calcium restored the secretory response to 60% of the initial output obtained in the absence of inhibitor. In other experiments, when 2 mM calcium was employed to evoke secretion instead of 0.5 mM, a given concentration of propranolol became less effective in depressing secretion and the propranolol inhibition curve was shifted to the right (Fig. 2c).

*The inhibition of the response to acetylcholine by local anaesthetics*

Experiments were then carried out to test the inhibitory effect of the local anaesthetics on the secretory response to ACh. The addition of $6\times10^{-6}$M ACh to normal Locke solution elicited catecholamine outputs of a similar order to those obtained with 0.5 mM calcium plus excess $K^+$, and thus some comparison could be made of the relative inhibitory effects of a given local anaesthetic on secretion evoked by ACh and calcium respectively.

Amethocaine produced a dose-dependent inhibition of the response to acetylcholine (Fig. 2a). The $ED_{50}$ of amethocaine in inhibiting the response to acetylcholine was estimated to be $4.2\times10^{-6}$M, which was tenfold lower than the $ED_{50}$ of amethocaine in blocking secretion elicited by calcium alone (Table 1). Cinchocaine and cocaine were also found to depress the response to ACh in low concentrations. However, there was little correlation between the concentration of either cinchocaine or cocaine and the degree of depression of the acetylcholine response. Thus, cinchocaine over the concentration range of $6\times10^{-6}$ to $5\times10^{-4}$M consistently produced an inhibition of the acetylcholine response of only about 50%. Similarly, increasing the concentration of cocaine over a range of $9\times10^{-6}$ to $9\times10^{-4}$M was ineffective in increasing the inhibition of the ACh response by more than 45%.

The ability of a given agent to inhibit ACh-evoked release varied widely from its activity in depressing calcium-induced secretion (Fig. 2a, b). This lack of correlation

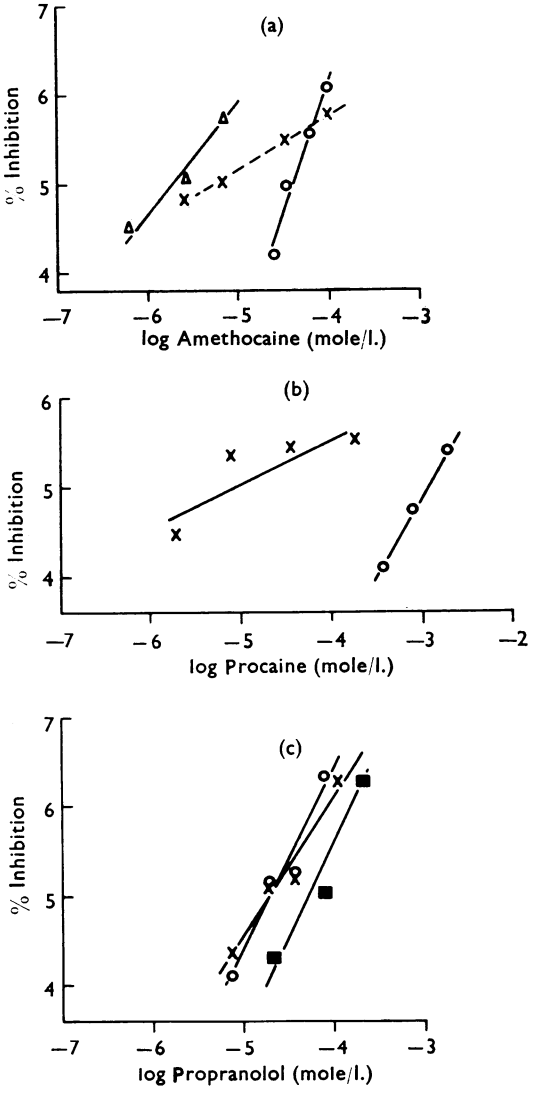


Fig. 2. Local anaesthetic inhibition of catecholamine secretion evoked by 0.5 mM calcium ○, 2 mM calcium ■, $6\times10^{-6}$ acetylcholine ×, and $1.2\times10^{-6}$ nicotine ▵. In experiments where calcium was used to elicit secretion, procedure was the same as described in Fig. 1. In experiments with acetylcholine and nicotine, glands were perfused with normal Locke solution and the stimulating agent was added for 2 min; perfusion was then switched to normal Locke solution plus inhibitor for 5 min and the 2 min stimulation repeated. Each point was obtained from a different preparation. See Methods for means of determining per cent inhibition, which is plotted in probit units.

was seen with low concentrations of amethocaine (Fig. 2a), as well as with cinchocaine and cocaine, but was most strikingly observed with procaine (Fig. 2b). Concentrations of procaine of $10^{-6}$M were sufficient to produce a significant inhibition of the response to acetylcholine, yet concentrations some 100 times higher were needed to inhibit calcium-

evoked secretion. Finally, in no instance was the inhibitory effect of a given local anaesthetic on the acetylcholine response even partially reversed by excess calcium (8–12 mM), which is in agreement with the results previously reported with amethocaine (Rubin *et al.*, 1967).

In the presence of cinchocaine or amethocaine, which produced about a 50% inhibition of the response to acetylcholine, a differential analysis of the catecholamine output showed that secretion was predominantly in the form of adrenaline (Fig. 3b, c). This apparent preferential inhibition of noradrenaline release by these local anaesthetics was strikingly similar to that seen with a dose of hexamethonium which also produced about a 50% depression of ACh-elicited secretion (Fig. 3a). Since it has been shown

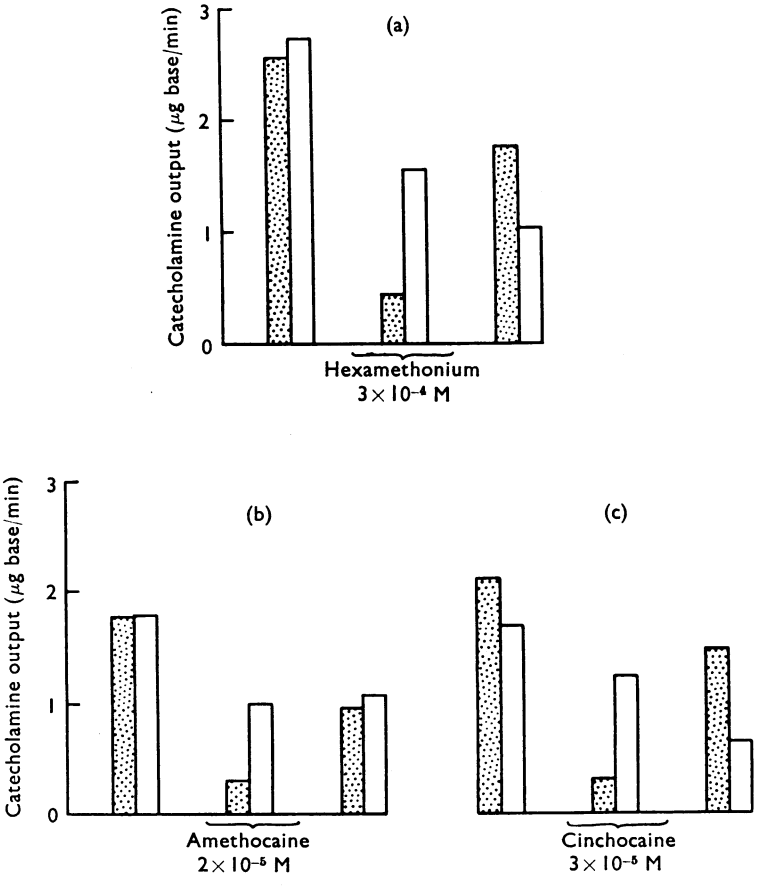


Fig. 3. Preferential inhibition of noradrenaline secretion by local anaesthetics. Glands were perfused alternately with normal Locke solution or Locke solution plus inhibitor for 7 min. During the last 2 min of each perfusion period, acetylcholine $6\times10^{-6}$ was added. The outputs of adrenaline (white column) and noradrenaline (dotted column) were determined during exposure to acetylcholine. The control rates of secretion just before the addition of acetylcholine are not shown but were usually $<0.2$ $\mu$g/min. (a), (b) and (c) are representative experiments each one carried out on a different preparation.

that specific stimulation of muscarinic receptors in the adrenal medulla of the cat leads to a predominance of adrenaline secretion (Douglas & Poisner, 1965), the preferential secretion of adrenaline observed in the presence of amethocaine and cinchocaine suggested that these local anaesthetics specifically blocked only the nicotinic actions of ACh. And indeed, the secretory response of a concentration of nicotine, which elicited outputs of the same magnitude as acetylcholine, was more markedly depressed by amethocaine than was the stimulating activity of acetylcholine (Fig. 2a). Cocaine also produced its inhibitory effect on acetylcholine-induced secretion by specifically suppressing noradrenaline release; however, procaine over the range of concentrations studied, inhibited both adrenaline and noradrenaline release to the same extent.

Propranolol showed a dose-dependent suppression of acetylcholine-evoked secretion which was not surmountable by increasing the calcium concentration six-fold. However, a close correlation was obtained in the case of propranolol between its inhibition of secretion evoked by acetylcholine and its inhibition of secretion evoked by calcium (Fig. 2c). The estimated $ED_{50}$ for inhibition by propranolol of the secretory response to ACh was identical to the $ED_{50}$ of propranolol in depressing calcium-evoked secretion ($3 \times 10^{-5}$ M). No specific block of the noradrenaline release was observed when propranolol was employed to depress the secretory response to ACh.

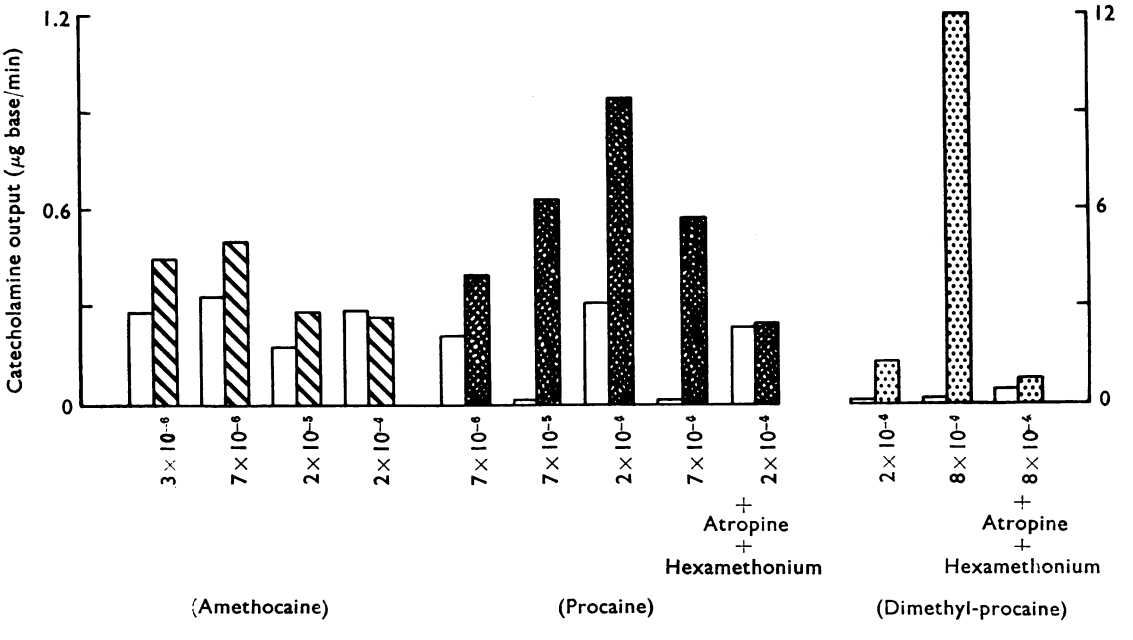


Fig. 4. Stimulation of catecholamine release by local anaesthetics. The clear vertical bars represent the control rates of secretion for the 2 min just before the addition of the local anaesthetic to the perfusion medium; whereas, the three types of shaded bars depict the rates of secretion during a 2 min exposure to the given agent. In 2 experiments atropine ($1.4 \times 10^{-5}$M) and hexamethonium ($3 \times 10^{-4}$M) were present for 5 min before procaine or dimethyl-procaine was added. The scale at the left applies to both amethocaine and procaine; the scale on the right applies only to the results obtained with dimethyl-procaine.

*The stimulant effects of local anaesthetics*

Since it appeared that some of the local anaesthetics could inhibit the response to acetylcholine by an action on acetylcholine-sensitive receptor sites, the catecholamine-releasing activity of the local anaesthetics was studied next, since it is well-known that receptor antagonists may sometimes also act as partial agonists. Amethocaine showed very small stimulating activity over a range of concentrations which did not inhibit calcium-evoked secretion (Fig. 4). Procaine proved to be a more potent stimulant and showed a graded release of catecholamines which reached a maximum of about 1 $\mu$g/min at a concentration of $2 \times 10^{-4}$M (Fig. 4). When the ethyl groups on the tertiary-nitrogen of the procaine molecule were replaced by methyl groups, the medullary stimulating activity was increased by more than tenfold (Fig. 4), although there was no significant change in the ability to depress secretion evoked by calcium. The increase in catecholamine output produced by procaine and its dimethyl analogue was abolished by a combination of atropine and hexamethonium which can completely suppress the response to acetylcholine ($6 \times 10^{-6}$M). The stimulant actions of these local anaesthetic agents appear to be the result of a direct action on the chromaffin cell and not due to release of acetylcholine from the cut splanchnic nerve-endings since dimethyl-procaine ($2 \times 10^{-4}$ M) elicited an output of 0.94 $\mu$g/min in a chronically denervated adrenal gland; this is comparable to the 1.36 $\mu$g/min evoked by this agent in an acutely denervated gland.

## DISCUSSION

The results of the present investigation have shown that agents which have local anaesthetic activity are able to block catecholamine secretion evoked by calcium. The site of action of the local anaesthetics is probably at the chromaffin cell membrane (see Rubin *et al.*, 1967). The inhibition is directed specifically against the calcium ion since the depression of calcium-evoked secretion by these agents could be overcome by excess calcium. Previous studies have shown that amethocaine can depress $Ca^{45}$ exchange in the adrenal gland (Rubin *et al.*, 1967), and can selectively block the inward calcium current in isolated chromaffin cells (Kanno & Douglas, 1967). The relative activity of each agent in inhibiting calcium-evoked secretion was estimated by dividing the $ED_{50}$ of procaine by the $ED_{50}$ of each of the other local anaesthetics, and the following molar ratios were obtained: cinchocaine 122; amethocaine 33; cocaine 11. These values for relative activities of the local anaesthetics compare reasonably well with activity studies of local anaesthetics on block of conduction in isolated nerve, haemolytic activity, irritancy, and systemic toxicity where the molar ratios relative to procaine ranged as follows: cinchocaine 13–120; amethocaine 9–37; cocaine 2–10 (Luduena, Hoppe & Borland, 1958; Andersen & Gravenstein, 1965; Truant & Takman, 1965). It thus appears that the inhibitory activity of the local anaesthetics at the adrenal medulla closely parallels their stabilizing activity in electrically excitable tissues such as muscle and nerve. The major difference appears to be the species of ions which are mainly affected. In the adrenal medulla, calcium appears to be the major focus of the inhibition produced by the local anaesthetics; in muscle and nerve, sodium and potassium are predominantly affected, although local anaesthetics are also able to block calcium movement in both skeletal and smooth muscle (Feinstein, 1963, 1966).

The present study also showed that the local anaesthetics could depress the secretory response to acetylcholine. The stimulant action of acetylcholine on the chromaffin cells is presumed to involve an increase in membrane permeability which allows calcium to enter the interior of the cell to initiate the secretion of catecholamines (Douglas & Rubin, 1961, 1963). Consistent with this hypothesis is the finding that acetylcholine requires the presence of calcium for its stimulating activity (Douglas & Rubin, 1961). In addition, it promotes the entry of $Ca^{45}$ into medullary cells (Douglas & Poisner, 1962), and high concentrations of amethocaine, which block this enhanced uptake of radiocalcium in the presence of acetylcholine, almost completely suppress catecholamine secretion (Rubin *et al.*, 1967). The inhibitory effect of amethocaine on acetylcholine-evoked secretion might be simply explained on the basis of a block of the calcium movement which is thought to follow the stimulation by acetylcholine. However, unlike the inhibition of catecholamine-secretion evoked by calcium with excess $K^+$, the inhibition of the acetylcholine-response by most of the local anaesthetics used in the present study did not show a clear-cut dose-response relationship. Only amethocaine showed a correlation between the depression of the acetylcholine response and the local anaesthetic concentration. Furthermore, there was no correlation between the efficacy of a given local anaesthetic to inhibit the response to acetylcholine and its ability to suppress calcium-evoked secretion. Procaine, which was about 100 times less potent than cinchocaine in blocking calcium-evoked secretion, was about as equally effective as cinchocaine in inhibiting the response to acetylcholine. Amethocaine was 10 times more effective in blocking the response to acetylcholine than the secretion elicited by calcium alone. These results suggest that low concentrations of a given local anaesthetic might block the acetylcholine response without interfering with calcium movement. Further investigations showed that amethocaine, cinchocaine, cocaine and procaine blocked the response to acetylcholine by actions on or near acetylcholine-sensitive receptor sites. This conclusion, which is not surprising in light of the structural similarity between the local anaesthetics used in this study and acetylcholine, is based upon the following pieces of evidence: (1) The inhibitory effect of the local anaesthetics on the acetylcholine response could not be antagonized by excess calcium (up to 6 times normal). (2) The blockade of the acetylcholine response by amethocaine, cinchocaine and cocaine manifested itself in a predominance of adrenaline secretion, just as did the block produced by hexamethonium, which suggests that in the presence of these local anaesthetics activation by acetylcholine was principally of muscarinic receptors (see Douglas & Poisner, 1965). (3) Amethocaine was more effective against the stimulating activity of nicotine than against that of acetylcholine, which unlike nicotine is presumed to activate muscarinic as well as nicotinic receptors. (4) Procaine and its dimethyl analogue proved to be effective stimulants of adreno-medullary secretion, and this stimulating activity was blocked by atrophine and hexamethonium. These results indicate that, in this system, certain local anaesthetics can act as partial agonists as well as antagonists.

It has been suggested by those who advance the hypothesis of a role for acetylcholine in nerve conduction (see Nachmansohn, 1959) that local anaesthetics block conduction by some action on an hypothesized acetylcholine-receptor in the axonal membrane (Higman & Bartels, 1961 ; Bartels & Nachmansohn, 1965). They have shown on the unicellular electroplax preparation that modification of the basic acetylcholine structure to that of certain local anaesthetics gradually transforms the molecule from an activator to an

inhibitor. Indeed, in the present study, dimethyl procaine still possessed strong catecholamine-releasing activity which was almost completely abolished by the addition of a butyl group to the aromatic ring to form amethocaine. Procaine, which possesses ethyl groups on the tertiary amino group, was less potent as a stimulating agent than its dimethyl analogue ; this finding is consistent with previous studies which have shown that tetraethylammonium (TEA) is a much less potent medullary secretogogue than tetramethylammonium (TMA) (Douglas & Rubin, unpublished observations).

Although the results of the present investigation support the notion that certain local anaesthetics because of their structural similarity to acetylcholine can interact with acetylcholine-sensitive sites, the significant point is that the block of the acetylcholine response could not be correlated with inhibition of calcium movement as measured in the adrenal medulla or with the ability of a given local anaesthetic to block nerve conduction. It is therefore extremely doubtful that the local anaesthetic activity of the analogues of acetylcholine depends upon the ability of these agents to react with an acetylcholine receptor as proposed by Nachmansohn and his associates. The conclusions of the present study are in agreement with an earlier study by Wiedling (1953), who found no connection between anti-acetylcholine potency of a number of local anaesthetics on guinea-pig ileum and local anaesthetic activity on the rabbit cornea. More recently, Feinstein and Paimre (1967) studying the inhibitory effect of amethocaine on acetylcholine-induced contraction of skeletal and smooth muscle, have concluded that the anti-cholinergic action of amethocaine is related to an antagonism of calcium in these tissues and not to any effect on drug-receptor interaction. The results of the present study have also shown that local anaesthetics can interfere with calcium movement, although unlike the muscle preparations, one can apparently demonstrate in the adrenal medulla an interaction of some of the local anaesthetics with an acetylcholine-sensitive site as well.

In addition to the classical local anaesthetics, propranolol was also found to be a potent inhibitor of secretion evoked by either calcium or acetylcholine. However, propranolol differed from the other local anaesthetics in showing a close parallelism between the block of the stimulant effects of both acetylcholine and calcium. This might suggest that propranolol was producing its inhibitory activity of the acetylcholine response solely by antagonizing calcium movement without having any direct effect on the interaction of acetylcholine with some receptor site. However, against this idea was the finding that excess calcium could reverse the inhibition by propranolol in the presence of excess $K^+$, but excess calcium was unable to reverse the inhibition when acetylcholine was employed as the stimulating agent. The notion that propranolol might act only on the flux of calcium without having any direct effect on the action of acetylcholine with its receptor is quite an appealing one, since propranolol, unlike the local anaesthetics employed in this study, does not possess a free tertiary amino group, and thus does not bear such a close resemblance to the acetylcholine molecule. In order to clarify this problem, additional studies are now being carried out utilizing other local anaesthetics which structurally do not resemble acetylcholine.

SUMMARY

1. Cat adrenal glands were perfused with Locke solution or calcium-free Locke solution containing excess $K^+$, and the inhibition by local anaesthetics of catecholamine output elicited by acetylcholine or calcium was studied.

2. Cinchocaine, amethocaine, cocaine and procaine caused a dose-dependent inhibition of catecholamine release produced by calcium, which could be reversed by increasing the calcium concentration. Their relative inhibitory activities on secretion correlated well with their relative local anaesthetic potency determined in other test systems.

3. These local anaesthetics also blocked the secretory response to acetylcholine. However, there was little correlation between the degree of block by a given local anaesthetic and its concentration in the perfusion medium. In addition, there was no correlation between the ability of these local anaesthetics to inhibit calcium-evoked secretion and secretion evoked by acetylcholine.

4. Procaine and its dimethyl analogue elicited increases in catecholamine output when added to normal Locke solution. These stimulant actions were blocked by hexamethonium and atropine.

5. Propranolol was more potent than amethocaine in depressing the secretory response to calcium. The inhibition by propranolol of the response to acetylcholine was well correlated with its ability to inhibit secretion evoked by calcium.

6. It is concluded that local anaesthetics can block calcium movement in the adrenal medulla in a manner which parallels their inhibition of monovalent cations in electrically excitable tissue. The block of the acetylcholine response by local anaesthetics is probably the result of both an inhibition of calcium movement and a direct action of these agents on acetylcholine-sensitive sites on the chromaffin cell membrane. However, the poor correlation between the ability of a local anaesthetic to inhibit calcium movement and its anticholinergic action casts doubt on the hypothesis that the effect of a local anaesthetic agent depends upon its interaction with an acetylcholine receptor.

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