Br. J. Pharmac. Chemother. (1966), **27**, 64–80.

THE EFFECT OF LOCAL ANAESTHETIC AND ANTI-ADRENALINE DRUGS ON THE RESPONSE OF SYMPATHETICALLY INNERVATED SMOOTH MUSCLE PREPARATIONS TO ELECTRICAL STIMULATION AT DIFFERENT FREQUENCIES

BY

G. A. BENTLEY

From the Department of Physiology, Monash University, Clayton, Victoria, Australia

(*Received November* 10, 1965)

Bentley (1965) has compared the ability of various drugs, including procaine, to block the response of the guinea-pig isolated vas deferens to electrical stimulation. In the course of related studies, it was noted that vas deferens preparations from rats and guinea-pigs showed certain differences in behaviour towards a number of substances (Sabine & Bentley, unpublished). Therefore it seemed appropriate to study the effects of procaine in more detail on several species, using various sympathetic nerve–smooth muscle preparations and to compare its action with that of other local anaesthetics and related substances.

METHODS

1. Vas deferens preparations

Stripped vas deferens preparations from young adult rats, guinea-pigs and rabbits were set up in organ baths of 80 ml. capacity, with a platinum electrode inserted in the lumen, as described by Bentley & Sabine (1963). They were suspended in a physiological saline solution of the following composition: sodium chloride, 120 mM ; potassium chloride, 5.0 mM ; calcium chloride, 2.5 mM ; sodium bicarbonate, 25 mM ; sodium dihydrogenphosphate, 1.0 mM ; magnesium sulphate, 1.0 mM ; glucose, 11.0 mM ; and sucrose, 10.0 mM. This was aerated with 95% oxygen–5% carbon dioxide. Electrical stimulation was applied from a Grass Model S4-D stimulator. Bursts of stimuli were used for 5 sec every 2 min. The frequency was alternated between 50/sec and 10, 15 or 20/sec and in each case the voltage was adjusted to give maximal contractions for the frequency used. Pulse duration was 0.5 msec. In most cases, the contraction was complete in less than 5 sec and the preparation had commenced to relax even though the stimulus was still on. However, in some cases, where guinea-pig or rabbit preparations were used the response to low frequency stimulation was still increasing after 5 sec stimulation, and in these cases stimulation was continued for 10 or 15 sec until relaxation commenced. A few experiments were also done using hypogastric nerve-vas deferens preparations (Huković, 1961) which were stimulated via the nerve as described above.

Further experiments were done with guinea-pigs to investigate the effects of prolonged low frequency stimulation. In a small proportion of cases it was found that if the stimulation at 15/sec was continued for 30–60 sec a second rise in tone occurred. Drug effects on this were also investigated.

Paired preparations of vas deferens were set up and stimulated as described above until responses to both frequencies were steady. The drugs under test were then added and allowed to act until responses were again steady. The bath was then washed out and the preparations were allowed to recover, after which higher concentrations could be tested if required. In some cases, drug levels in the bath were increased without washing out.

2. Intestinal preparations

Preparations of rabbit ileum with a length of mesenteric artery attached were set up as described by Finkleman (1930), with the artery held between annular platinum electrodes. Stimulation of the periarterial nerves was carried out at frequencies of 10/sec for 30 sec and 50/sec for 20 sec alternately each 3 min. Voltage was adjusted so that a large but not maximal inhibition occurred. Responses were recorded using a frontal-writing lever on a smoked drum.

3. Isolated rabbit ear artery preparations

Some experiments were also done using the isolated central artery of the rabbit ear (Delalande & Rand, 1965). Rabbits were anaesthetized with intravenous injections of 25% urethane. The central artery was exposed for a short distance at the base of the ear and a polythene cannula inserted. A length of the artery about 5 cm long was cut out, still enclosed in skin and placed in an organ bath at 37° C. This was perfused with conventional Krebs solution from a Julian Smith blood pump with a mercury manometer connected. Fine platinum wires insulated to within 1 cm of the terminals were attached to the artery just distal to the cannula, supplying electrical stimulation from the Grass stimulator. Stimuli of 0.5 msec pulse duration were applied at rates of 5 and 25/sec alternately for periods of 15 or 20 sec each 3 min. Voltage was adjusted to give a large but not maximal response, and the increases in pressure were recorded on a smoked drum.

4. Interaction of local anaesthetics with noradrenaline

Dose-response curves to noradrenaline on rat and guinea-pig vas deferens were prepared as described previously (Bentley, 1965) and then repeated in the presence of the local anaesthetics. Similar experiments were also done, using isolated rabbit ileum. Doses of noradrenaline were adjusted so that responses in the presence of the local anaesthetics matched the control contractions. It is difficult to prepare accurate dose-response curves to noradrenaline on vas deferens preparations, especially when guinea-pig tissue is used, as responses are seldom closely reproducible. Therefore no attempt was made to calculate accurately the changes in sensitivity caused by the local anaesthetics, but these were estimated from graphs of the dose-response curves. Standard errors of these approximate alterations in sensitivity were calculated to give an indication of the variability of this effect.

5. Drugs used

Amphetamine sulphate (B.D.H.); Atropine sulphate (T. & H. Smith); Cocaine hydrochloride (Drug Houses of Australia); Physostigmine sulphate (T. & H. Smith); Iproniazid phosphate (Hoffman La Roche); Lignocaine hydrochloride (Xylocaine, Astra); Phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline & French); Phentolamine hydrochloride (Rogitine, Ciba); Piperoxane hydrochloride (May & Baker); Prilocaine hydrochloride (Citanest, Astra); Procaine hydrochloride (Bull Laboratories); Yohimbine hydrochloride (Andrews Laboratories).

RESULTS

1. Guinea-pig vas deferens

The transmurally stimulated guinea-pig vas deferens always responded with a much larger contraction to stimulation at 50/sec than to the lower frequency, and often the effect of the 10/sec stimulation was very small, or showed marked reduction with time. Consequently 15/sec was mostly used, and on a few occasions 20/sec was necessary.

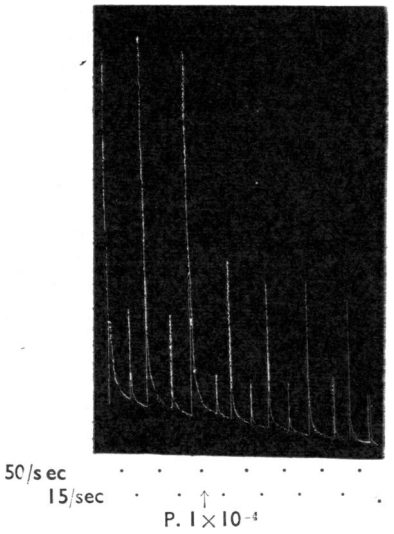

Fig. 1. Guinea pig vas deferens, transmural stimulation alternately at 15 and 50/sec, for 5 sec each 2 min. Stimuli applied at dots. At P, procaine added to 1×10^{-4}.

TABLE 1

EFFECTS OF PROCAINE, LIGNOCAINE AND COCAINE ON RESPONSES OF VAS DEFERENS PREPARATIONS TO ELECTRICAL STIMULATION AT HIGH AND LOW FREQUENCIES AND TO NORADRENALINE

Drug	Rat			Guinea-pig		
	Electrical Stimulation 50/sec	10/sec	Noradren. potentiation	Electrical Stimulation 50/sec	15/sec	Noradren. potentiation
Procaine 1×10^{-4}	Decrease	Increase	$4{\cdot}2\pm0{\cdot}7\times$	Decrease	Decrease	$2{\cdot}9\pm0{\cdot}6\times$
Lignocaine 5×10^{-5}–1×10^{-4}	Decrease	Decrease	$1{\cdot}7\pm0{\cdot}4\times$	Decrease	Increase	$9{\cdot}8\pm3{\cdot}2\times$
Cocaine 1×10^{-5}	Slight Increase	Decrease	$40{\cdot}0\pm8{\cdot}2\times$	Decrease	Decrease	$31\pm5{\cdot}8\times$

TABLE 2

EFFECTS OF VARIOUS DRUGS AND OF LOWERED TEMPERATURE ON RESPONSES OF RAT AND GUINEA-PIG VAS DEFERENS TO ELECTRICAL STIMULATION AT HIGH AND LOW FREQUENCIES

Treatment	Rat		Guinea-pig	
	50/sec	10/sec	50/sec	15/sec
Low temperature (25°C)	Slight Increase	Slight Increase	Slight Increase	Increase
Prilocaine 1×10^{-4}	Decrease	Slight Increase	Decrease	Decrease
Physostigmine 1×10^{-6}–1×10^{-5}	Increase	Increase	Increase	Increase
Yohimbine 1×10^{-6}–1×10^{-5}	Decrease	Increase	Decrease	Decrease
Phentolamine 1×10^{-5}	Usually Decrease	Usually Increase	Usually Decrease	Usually Decrease
Phenoxybenzamine 1×10^{-6}–1×10^{-5}	Decrease	Decrease	Increase	Increase
Piperozane 1×10^{-6}–1×10^{-5}	Slight Decrease	Slight Increase	Increase	Increase

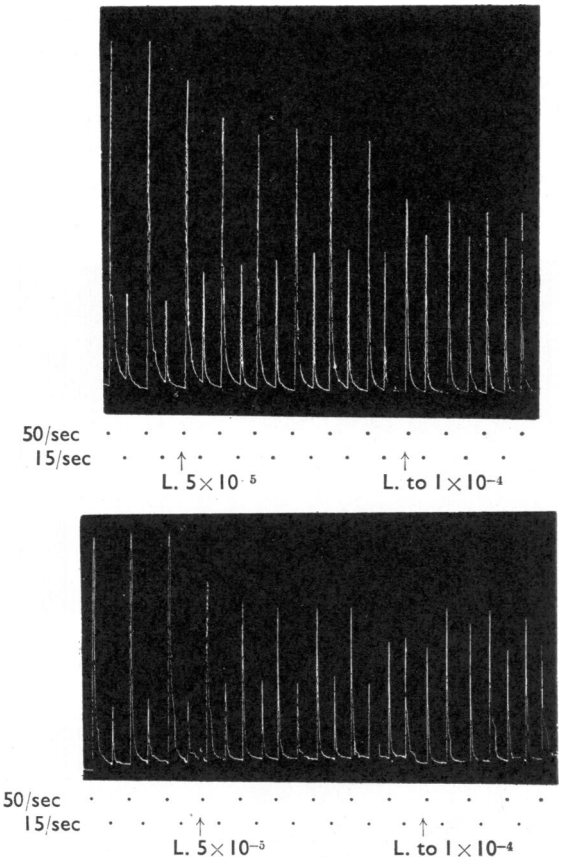

Fig. 2. Guinea pig vas deferens, stimulation as in Fig. 1. At L, 5×10^{-5}, lignocaine added to 5×10^{-5}, and at L, 1×10^{-4}, the concentration of lignocaine was increased to 1×10^{-4}. Note progressive reduction in response to 50/sec stimulation, and increase in 15/sec responses. In lower panel, the latter responses became larger than those to 50/sec stimulation.

Procaine at levels in the bath of $1–2\times10^{-4}$ markedly depressed the responses to both fast and slow stimulation (Fig. 1). Usually the percentage reduction in response to 50/sec was a little greater than to the lower rates. Higher concentrations of the drug completely and rapidly blocked both responses. In all cases the drug-induced depression was readily reversed by washing.

When lignocaine $5\times10^{-5}–1\times10^{-4}$ was used, quite different results were obtained. In most cases the response to 50/sec stimulation was depressed to varying degrees, while that to slow stimulation was almost always increased. This increase in low frequency responses could amount to a doubling of the original height of contraction (Fig. 2). The effect usually developed slowly, and was particularly marked if the drug was washed out and immediately replaced. Higher doses of lignocaine (5×10^{-4}, 1×10^{-3}) had various effects. Occasionally complete block occurred, but often a very large potentiation of both responses was seen. In yet other cases the effect of electrical stimulation was obscured by large spontaneous contractions.

Where preparations were stimulated via the hypogastric nerve, similar results were obtained in most cases. i.e., a depression of both responses with procaine, and an increase in the low frequency response plus depression of the effects of 50/sec stimulation in the presence of lignocaine. However, in about 50% of cases, lignocaine at 5×10^{-5}–1×10^{-4} completely abolished all responses, presumably because of a block in the ganglion (see Bentley & Sabine, 1963), as sympathetic ganglia are known to be susceptible to block by local anaesthetics (Harvey, 1939).

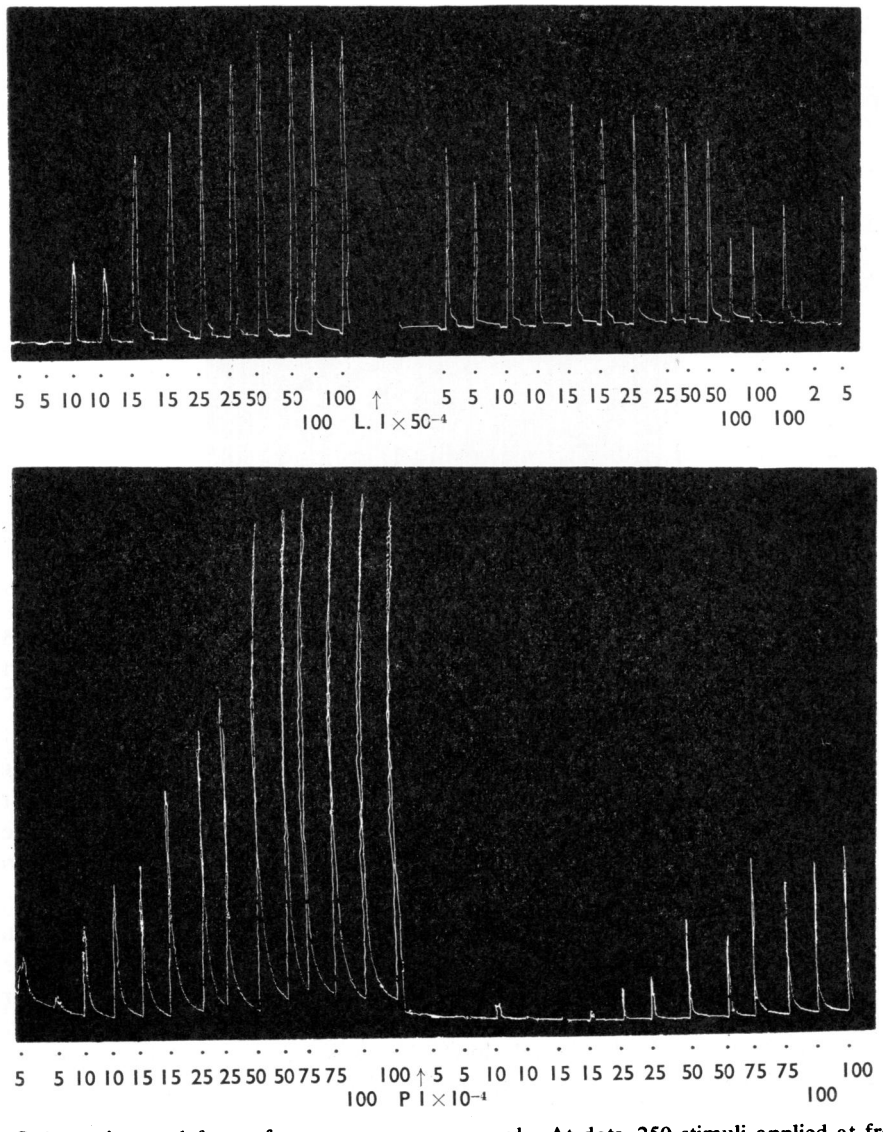

Fig. 3. Guinea pig vas deferens frequency-response record. At dots, 250 stimuli applied at frequency indicated below. Upper panel: At L, lignocaine added to 1×10^{-4}, and after 10 min wait, stimulation repeated again. Lower panel: At P, procaine added to 1×10^{-4}.

Cocaine, $1–5 \times 10^{-5}$ in most cases acted like procaine on the guinea-pig, depressing both fast and slow stimulus responses, and prilocaine $1 \times 10^{-6}–1 \times 10^{-4}$ also produced similar effects (see Tables 1 and 2).

Because lignocaine had such a different effect on responses at 10–15/sec and at 50/sec it seemed important to investigate the actions of both lignocaine and procaine over a wide range of stimulus frequencies. Accordingly, control responses to 250 stimuli at 5, 10, 15, 25, 50, 75 and 100/sec were recorded, then the preparations were treated with 1×10^{-4} lignocaine or procaine, and the responses were recorded again. It was found that procaine depressed responses at all frequencies, while lignocaine potentiated responses at 5, 10 and 15/sec, had little effect on 25/sec and depressed all the higher frequency responses by about the same amount (Fig. 3). At higher levels of lignocaine (5×10^{-4}) the response to all frequencies was greatly increased, except where large spontaneous spasms occurred which completely obscured any responses to electrical stimulation. At 1×10^{-3} lignocaine abolished any spontaneous contractions, and completely blocked all responses to electrical stimuli.

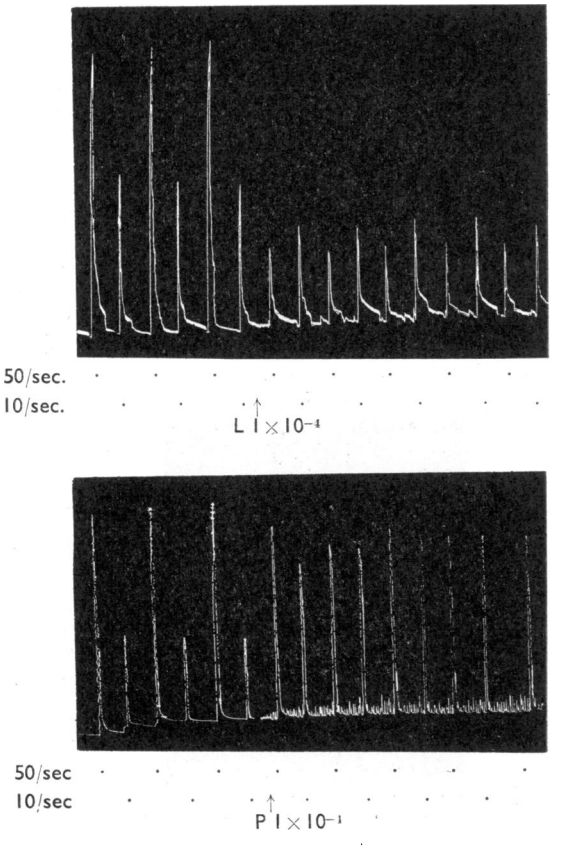

Fig. 4. Rat vas deferens, transmural stimulation at 10 and 50/sec alternately. Upper panel: At L, lignocaine added to 1×10^{-4}. Both responses depressed. Lower panel: At P, procaine added to 1×10^{-4}. Responses to 50/sec stimulation depressed, and to 10/sec, increased. Some spontaneous activity also apeared in presence of procaine.

2. Rat

The rat vas deferens responded well to stimuli at 10/sec and the contractions so produced were usually only a little smaller than those in response to 50/sec.

In this species the effects of the local anaesthetics differed from those in the guinea-pig. Procaine, $1–2 \times 10^{-4}$ depressed the responses to 50/sec stimulation but potentiated those to 10/sec, while lignocaine, $5 \times 10^{-5}–1 \times 10^{-4}$ depressed both responses (Fig. 4). Cocaine $5 \times 10^{-6}–1 \times 10^{-5}$ had various effects. It always depressed the low frequency responses, but either did not affect responses at 50/sec or slightly increased them (Fig. 5). At higher doses, marked depression of all responses occurred.

Prilocaine on the rat produced effects similar to procaine, depressing responses to stimulation at 50/sec, at levels in the bath of $1 \times 10^{-6}–1 \times 10^{-4}$, but slightly increasing the responses to low frequency stimulation up to levels of 5×10^{-5}. At 1×10^{-4}, these low frequency responses were also depressed (see Tables 1 and 2).

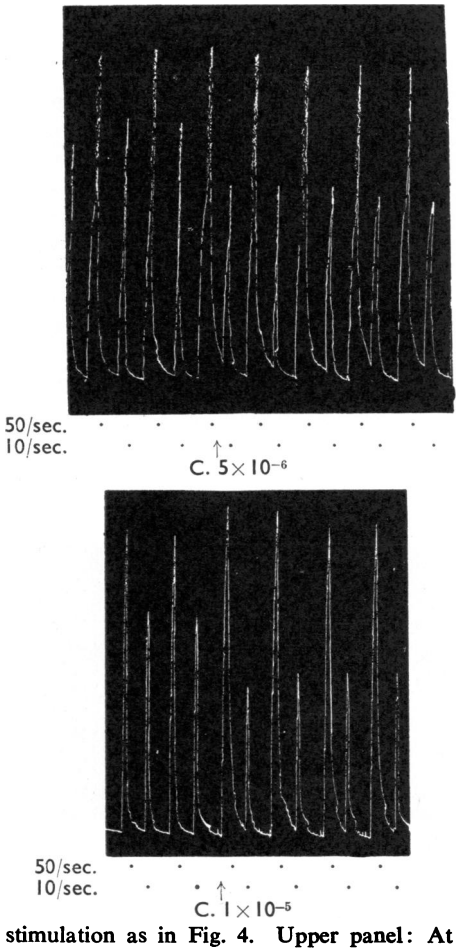

Fig. 5. Rat vas deferens, stimulation as in Fig. 4. Upper panel: At C, Cocaine added to 5×10^{-6}. Lower panel: At C, Cocaine added to 1×10^{-5}. Responses to low frequency stimulation are depressed in both cases, but to 50/sec stimulation are either scarcely affected or slightly increased.

3. Rabbit

(a) Vas deferens

The rabbit vas deferens behaved like that of the guinea-pig, giving very small responses to stimuli at 10/sec. At 15/sec the contractions were larger, but sometimes fell off as time progressed. This species was rather more sensitive to the depressant action of local anaesthetics, as procaine and lignocaine at $1–5 \times 10^{-5}$ and cocaine 1×10^{-5} all depressed the responses to both 15 and 50/sec stimulation.

(b) Finkleman preparation

When inhibitory sympathetic responses of the rabbit ileum Finkleman preparation were studied, neither procaine nor lignocaine, $1–5 \times 10^{-5}$, gave any suggestion of increasing the effects of 10/sec stimulation, though in most cases these were depressed to a lesser extent than the effects of 50/sec stimulation (see Table 3).

TABLE 3

RABBIT EAR ARTERY, VAS DEFERENS AND FINKLEMAN PREPARATIONS. EFFECTS OF PROCAINE, LIGNOCAINE AND COCAINE ON HIGH AND LOW FREQUENCY ELECTRICAL STIMULATION

	Rabbit Ear Artery		Rabbit vas deferens		Rabbit Ileum Finkleman Preparation	
Drug	25/sec	5/sec	50/sec	15/sec	50/sec	10/sec
Procaine 1×10^{-5}–1×10^{-4}	Large Decrease	Small Decrease	Decrease	Decrease	Decrease	Small Decrease
Lignocaine 1×10^{-5}–1×10^{-4}	Large Decrease	Small Decrease	Decrease	Decrease	Decrease	Small Decrease
Cocaine $1–5 \times 10^{-7}$	Increase	Increase	—	—	—	—
$1–5 \times 10^{-6}$	Decrease	Decrease	—	—	—	—
$1–5 \times 10^{-5}$	—	—	Decrease	Decrease	Decrease	Small Decrease

(c) Rabbit ear artery

Electrical stimulation of the isolated perfused rabbit ear artery caused increases in the perfusion pressure. The effect of stimulation at 5/sec was usually not much smaller than at 25/sec. Procaine 1×10^{-5}–1×10^{-4} and lignocaine 1×10^{-5}–1×10^{-4} both caused only a small depression of the responses to 5/sec stimuli, but were much more effective against the faster rate, causing complete block at the higher concentrations. Cocaine at $1–5 \times 10^{-7}$ either had no effect or increased both responses, but at higher concentrations ($1–5 \times 10^{-6}$) depression of both occurred. After this drug was washed out recovery rapidly occurred, often to levels considerably higher than the control (see Table 3).

4. Effects of lowered temperature

Shanes (1958) has classified local anaesthetics as " stabilizers " of the cell membrane, and has noted that low temperature has similar effects. Della Bella, Gandini & Preti (1965) have studied the effects of low temperature on the response to stimulation of the guinea-pig vas deferens via the hypogastric nerve. In view of the findings noted above that rat and guinea-pig vas deferens respond differently to drugs it seemed advisable to compare the effects of low temperature (25° C) also on both species.

It was found that at 25° C the contractions to stimulation at the lower frequency were much slower than at 35° C, in both species, though the speed of the 50/sec stimulation

response was not noticeably changed. Where necessary, the period of stimulation was prolonged until the contraction was maximal and relaxation had commenced. On the guinea-pig, results obtained in the present investigation differed slightly from those of Della Bella *et al.* (1965) in that low temperature either had no effect on or slightly increased the responses to 50/sec stimulation. However, results with the low frequency stimulation were similar to those of the above authors, i.e., these were markedly increased at the lower temperature. The rat responded to low temperature very similarly to the guinea-pig in that both responses were increased, though the effect on the low frequency response was much less marked than in the guinea-pig (see Table 2).

5. Interactions with atropine and amphetamine

Bentley (1965) has shown that amphetamine 1×10^{-6} added to a vas deferens already treated with procaine 1×10^{-4} will cause a further 2–3-fold increase in sensitivity to noradrenaline. Amphetamine was therefore used in an attempt to alter the effects of procaine on the electrically stimulated guinea-pig vas deferens. It was found that the local anaesthetic depressed both the high and low frequency responses equally well in the presence or absence of amphetamine.

Similarly it was found that atropine 1×10^{-6} did not alter the ability of lignocaine to potentiate low frequency responses, suggesting that this phenomenon is not due to a cholinergic mechanism.

6. Effects of eserine and anti-adrenaline drugs

Bülbring (1946) reported that physostigmine on the rat phrenic nerve diaphragm preparation depressed responses to high frequency, but potentiated the effects of low frequency stimulation, and Burn & Weetman (1963) had noted a similar effect of eserine plus atropine on the guinea-pig hypogastric nerve-vas deferens preparation. On the other hand, Della Bella, Benelli & Gandini (1964) showed a potentiating effect of physostigmine on both high and low frequency stimulation. Boyd, Chang & Rand (1960) had also noted that several anti-adrenaline drugs potentiated the response of the guinea-pig hypogastric nerve-vas deferens preparation to electrical stimulation at a frequency of 10/sec. It seemed important therefore to test physostigmine and various anti-adrenaline drugs on both rat and guinea-pig vas deferens, especially in view of the fact that procaine and to a lesser extent lignocaine both have anticholinesterase activity (Adler, Gal & Vegh, 1950). Furthermore, yohimbine, one of the anti-adrenalines tested by Boyd *et al.* is a potent local anaesthetic (MacCallum, McCallum & Shaw, 1949).

It was found that physostigmine 1×10^{-5} to 1×10^{-6} potentiated responses to both high and low frequency stimulation on both rat and guinea-pig preparations. The average percentage increase in high frequency response was approximately equal for both species, but with low frequency stimulation the increase in size of responses was greater with guinea-pig than with rat, perhaps because in the former species these control responses were usually small. The potentiating action of physostigmine increased as the concentration was raised from 1×10^{-6} to 1×10^{-5} and also was greater where stimulation was via the hypogastric nerve than with transmural stimulation.

Atropine, 1×10^{-6} added 20–30 min after physostigmine, completely reversed the potentiating action of physostigmine in both species causing both high and low frequency

responses to return to just below control levels within a few min (Fig. 6). If atropine was added before the physostigmine, it prevented any potentiation from occurring. This antagonism between physostigmine and atropine was seen with both hypogastric and transmural stimulation.

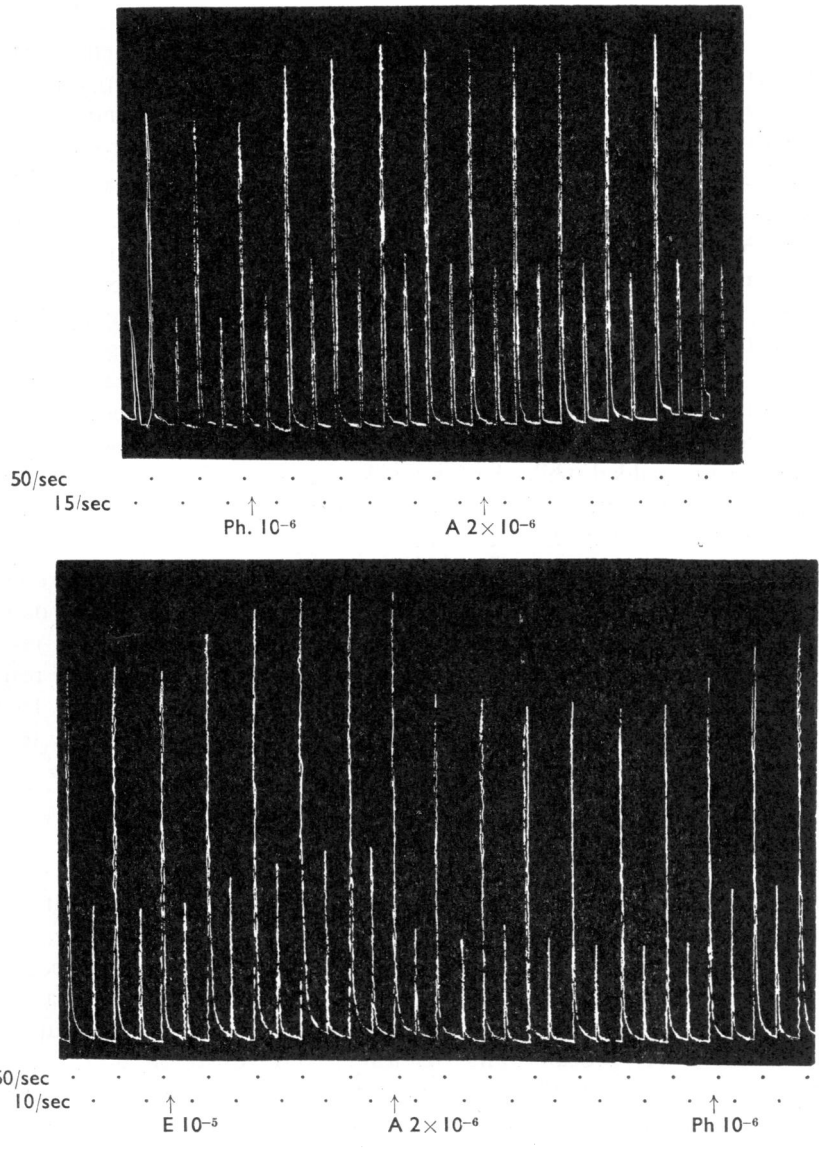

Fig. 6. Guinea-pig vas deferens, stimulation as in Fig. 1. Upper panel: At Ph 1×10^{-6}, phenoxybenzamine added to 1×10^{-6}, at A, 2×10^{-6}, atropine added to 2×10^{-6}. Lower panel: At E 1×10^{-5}, physostigmine added to 1×10^{-5}, at A, 2×10^{-6} atropine added to 2×10^{-6}, and at Ph 1×10^{-6}, phenoxybenzamine added to 1×10^{-6}. The potentiating action of physostigmine, but not of phenoxybenzamine, was reversed by atropine.

Phenoxybenzamine was tested on rat and guinea-pig preparations and, as with the local anaesthetics, a marked species difference was noted. On the guinea-pig, this drug at levels of 1×10^{-6} produced a very marked increase in responses to both fast and slow stimulation. This increase was much greater than that caused by physostigmine at 1×10^{-5}, and, unlike the action of the latter drug, the potentiation caused by phenoxybenzamine was completely unaffected by atropine 1×10^{-6} to 1×10^{-5}, added either before or after the phenoxybenzamine (Fig. 6). At 1×10^{-5} phenoxybenzamine caused a small further increase in both responses, but at 1×10^{-4}, the preparation showed a progressive depression to almost complete block. At 1×10^{-5} and above some spontaneous activity developed sometimes, which was not affected by atropine.

On the rat phenoxybenzamine caused a weak depression of both responses at levels of 1×10^{-6} to 1×10^{-5}, and at 1×10^{-4} a marked depression occurred.

Phentolamine produced quite different effects. On guinea-pig preparations, at 1×10^{-6} to 1×10^{-5}, the most usual result was a depression of fast and slow responses, though occasionally the responses to stimulation at 15/sec were either not affected or slightly increased. On the rat its effect was quite variable. It usually depressed responses to fast stimulation, but increased those to 10/sec stimulation. However, increases or decreases in both responses were also observed occasionally (see Table 2).

Where phentolamine had slightly depressed the low frequency responses in guinea-pig preparations, lignocaine, added while the former drug was still present, showed its usual ability to increase low, and depress high, frequency responses, and similarly, phentolamine did not alter these effects of lignocaine after they had appeared.

The third anti-adrenaline drug tested, yohimbine, also showed species differences. At levels of 1×10^{-6} to 1×10^{-5} on guinea-pig preparations it caused a marked depression of both high and low frequency responses. However, on the rat its action resembled procaine in that species, increasing the low, and decreasing the high frequency responses.

Piperoxane had different actions again. At concentrations of 1×10^{-6} to 1×10^{-5} it resembled phenoxybenzamine on the guinea-pig, increasing both high and low frequency responses, but on the rat it decreased high, but augmented the low frequency responses (see Table 2).

7. Effects on prolonged low frequency stimulation

The presence of a biphasic response in some guinea-pig vas deferens preparations when prolonged low frequency stimulation was used suggested the possibility that there was some diffusion of transmitter to more distant receptors. The drug-induced potentiation of the low frequency responses could have been due to an increased amount of transmitter reaching these more distant receptors, perhaps due to diminished uptake into storage sites. Accordingly procaine and lignocaine were tested on preparations that showed these biphasic responses and were compared with other drugs known to interfere with noradrenaline storage (amphetamine, cocaine) or to prevent its metabolism (iproniazid). Since Della Bella, Benilli & Gandini (1964) had suggested that a cholinergic mechanism is present in the vas deferens, atropine was also tested.

It was found that lignocaine potentiated the first, but depressed the second component of the prolonged response, while procaine depressed both components (Fig. 7). The effects of the other drugs are set out in Table 4.

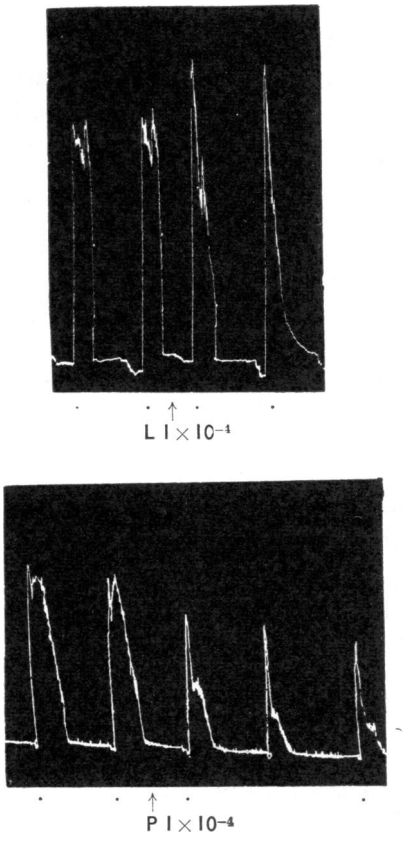

Fig. 7. Guinea pig vas deferens. Stimulation at 15/sec for 45 sec each 8 min. Upper panel: At L, lignocaine added to 1×10^{-4}. Lower panel: At P, procaine added to 1×10^{-4}. Lignocaine increases the early response, decreases the later one. Procaine depresses both early and late responses.

TABLE 4

GUINEA-PIG VAS DEFERENS. EFFECT OF LOCAL ANAESTHETICS AND OTHER DRUGS ON RESPONSE TO PROLONGED STIMULATION AT FREQUENCY OF 15/SEC

Treatment		Effect on First Component	Effect on Second Component
Lignocaine	5×10^{-5}	Increase	Decrease
Procaine	5×10^{-5}	Decrease	Decrease
Amphetamine	1×10^{-6}	Marked Increase	No Change
Cocaine	1×10^{-5}	Decrease	Increase
Iproniazid	1×10^{-5}	No Change	No Change
Atropine	1×10^{-6}	No Change	No Change
	1×10^{-5}	Decrease	Decrease

8. *Interaction with noradrenaline*

It seemed possible that potentiation of slow and depression of fast stimulation responses could result from a prolongation of the refractory period of the nerve plus a potentiation of the effects of liberated transmitter. Accordingly, experiments were performed to test

the ability of procaine, lignocaine and cocaine to alter the sensitivity of rat and guinea-pig vas deferens, and of rabbit ileum to added noradrenaline.

Bentley (1965) has shown that procaine 1×10^{-4} potentiates noradrenaline on guinea-pig vas deferens by a factor of 2.9 ± 0.6 times. In the present investigation it was found that lignocaine 5×10^{-5} was a more effective potentiator in this species (9.8 ± 3.2 times) and cocaine, as might be expected from its effects on other tissues, was still more effective (31 ± 5.8 times).

When rat was used, however, different results were obtained. Procaine caused more potentiation than in the guinea-pig (4.2 ± 0.7 times) while lignocaine only slightly increased sensitivity to noradrenaline (1.7 ± 0.4 times). Cocaine, however, was even more effective than that on the guinea-pig (40 ± 8.2 times) (see Table 1).

Using rabbit ileum, no exact estimate could be made as the local anaesthetics reduced the contractions to varying degrees. However, all three drugs increased the sensitivity of this tissue to about the same extent, namely, about two-fold.

9. Interactions with magnesium

It was found that if magnesium was omitted from the physiological saline, lignocaine had much less depressant action on the responses of the guinea-pig vas deferens to stimulation at 50/sec and potentiated responses to 10/sec stimulation even more than when magnesium was present. On the other hand, the potentiating effects of physostigmine were not apparently affected by magnesium levels.

DISCUSSION

The experiments described in this paper have produced several unexpected results. For example, it is surprising that depressant drugs such as local anaesthetics should cause potentiation of certain nervously mediated responses. It is also surprising that this potentiation can be seen only on rat and guinea-pig vas deferens and on rabbit ear artery, but not on rabbit vas deferens or intestine. Bentley & Sabine (unpublished) have noted other differences in sympathetically mediated responses of rat, rabbit and guinea-pig tissues.

It would be convenient if one could explain the potentiation by local anaesthetics of the responses to low frequency stimulation as being due to a potentiation of the sympathetic transmitter. If the refractory period of the nerves was at the same time increased the consequent reduction in output of transmitter at high frequency stimulation would explain why these responses were depressed. Della Bella, Gandini & Preti (1965) explained their findings on the effect of cold on sympathetically induced responses on the grounds of increased refractory period. Unfortunately, however, this is not adequate to explain the present results, for the following reasons:

(i) The ability of the local anaesthetics to potentiate added noradrenaline does not correlate with their effect on the low frequency responses. For example, cocaine is most effective in potentiating added noradrenaline on both rat and guinea-pig, yet it does not increase low frequency responses in either species. Also procaine, whose ability to potentiate noradrenaline on the rat is only slightly greater than on the guinea-pig, depresses low frequency responses on the latter species, but potentiates them on the rat.

(ii) Amphetamine, which has been shown to cause a 2–3-fold potentiation of noradrenaline in the presence of procaine (Bentley, 1965) does not alter the depressant action of procaine on the response of the guinea-pig vas deferens to low frequency electrical stimulation. Furthermore, where lignocaine has caused potentiation of the low frequency responses in guinea-pig preparations, the addition of the adrenolytic drug phentolamine does not remove this potentiation.

(iii) Yohimbine, which has both potent local anaesthetic and anti-adrenaline activities, increases responses to stimulation at 10/sec in the rat, but not in the guinea-pig. In contrast, phenoxybenzamine, another potent anti-adrenaline, potentiates low frequency responses in the guinea-pig, but not in the rat. This effect on the guinea-pig agrees with the findings of Ohlin & Strömblad (1963) but not of Della Bella, Benelli & Gandini (1964) who reported a depressant action of phenoxybenzamine. The potentiating action of the anti-adrenaline drug, unlike that of physostigmine, was not abolished by atropine.

(iv) Procaine, lignocaine and cocaine all approximately double the sensitivity of the rabbit ileum to noradrenaline, yet they do not potentiate the effects of low frequency electrical stimulation applied to the periarterial nerves.

Similarly, it seems unlikely that the effects of the local anaesthetics involves cholinergic mechanisms, despite the findings of Boyd, Chang & Rand (1960) for the following reasons:

(a) Procaine has 10 times the anticholinesterase potency of lignocaine (Adler *et al.*, 1950) yet it does not increase low frequency responses in the guinea-pig vas deferens, which is many times more senstive to cholinomimetic drugs than is the rat.

(b) After procaine or lignocaine have increased low frequency responses in the rat or guinea-pig respectively, the addition of atropine 1×10^{-6} does not alter this effect. Della Bella, Gandini & Preti (1964) have shown that the ability of physostigmine to increase both high and low frequency responses in guinea-pig vas deferens is abolished by atropine, and this has been confirmed in the present paper.

The occasional appearance of a double response in a guinea-pig vas deferens after prolonged low frequency stimulation suggested that released transmitter might be diffusing to more distant sites of action. If this were so, the potentiation of the low frequency responses by local anaesthetics could have resulted from an ability of these substances either to facilitate the diffusion of transmitter or to increase the sensitivity of the receptors further from the nerve endings. However, this explanation seems unlikely, since lignocaine, phentolamine and procaine all depressed the second component of the prolonged response, although the first two drugs increased the size of the first response on guinea-pig preparations.

The lack of correlation between the ability of the local anaesthetics to potentiate added noradrenaline and to increase the size of the response to low frequency stimulation argues against the theory that this latter effect depends on a sensitization of the smooth muscle to the transmitter. However, before this is entirely dismissed, it must be pointed out that the receptors acted on by added substances may not be the same as those activated by transmitter substance from the nerve, and in fact may behave differently. Further studies are in progress to investigate drug effects on cell membrane changes, using the sucrose gap apparatus.

The low potency of anti-adrenaline drugs against electrically induced responses as compared with their activity against added noradrenaline remains puzzling. Dale & Gaddum (1930) had remarked on a similar phenomenon where atropine was ineffective in blocking the effects of vagal stimulation on the stomach, and Boyd, Chang & Rand (1960) had described the ability of several anti-adrenaline drugs to potentiate the response of the guinea-pig hypogastric nerve-vas deferens preparation to electrical stimulation at 10/sec. They attributed this effect to the anti-cholinesterase action of the anti-adrenaline drugs. Ohlin & Strömblad (1963) however, disagreed with their findings, and further reasons for doubting this explanation are presented in this paper, since firstly the potentiating action of the anti-adrenaline drugs varied according to whether rat or guinea-pig vas deferens is used. Secondly, atropine did not alter this potentiating action of the anti-adrenaline drugs though it completely abolished the action of physostigmine tested under identical conditions.

The effects of physostigmine warrant further comment. Burn & Weetman (1963) using isolated preparations of the guinea-pig hypogastric nerve-vas deferens showed that this drug in the presence of hyoscine or atropine markedly potentiated responses to stimulation at 5 or 10/sec, but slightly reduced the effects of stimulating at 40/sec. However, in the present paper different results were obtained, and it was found that atropine, 1 μg/ml., abolished the potentiating action of physostigmine on both the responses to 15/sec and 50/sec stimulation. This effect was seen irrespective of whether the preparation was stimulated via the hypogastric nerve, or transmurally. These findings confirm the results of Della Bella, Gandini & Preti (1964). They are in marked contrast to the effects of both anti-adrenaline and local anaesthetic drugs, whose potentiating action is not affected by atropine, added either before or after the other drug. No explanation can be advanced for the discrepancy between the results of Burn & Weetman on the one hand, and of Della Bella *et al.*, and of the present paper, on the other.

The findings reported in the present paper still lack an adequate explanation. Nevertheless they underline the risks inherent in extrapolating results from one species to another, or in assuming that drugs apparently as similar as procaine and lignocaine will act identically in simple biological systems.

SUMMARY

1. The local anaesthetic drugs procaine, lignocaine, cocaine and prilocaine, have been examined for their ability to affect the responses of isolated preparations of rabbit, rat, and guinea-pig vas deferens to electrical stimulation at fast (50/sec) and slow (10–15/sec) rates.

2. With the exception of cocaine acting on rat preparations, all these drugs depressed the responses to fast stimulation. However, slow stimulation responses could be either depressed or augmented, according to the drug and the species used. This effect was not altered by atropine.

3. When Finkleman preparations of rabbit ileum, or perfused rabbit ear arteries were used, responses to slow frequency stimulation were depressed by the local anaesthetics to a lesser extent than were those to fast stimulation, though augmentation was not seen except in the case of cocaine on the artery preparation, where low concentrations augmented both responses.

4. Rat and guinea-pig vas deferens preparations were also used to test the effects of the anti-adrenaline drugs phenoxybenzamine, phentolamine, piperoxane and yohimbine. As with the local anaesthetics, these drugs also produced either augmentation or depression of the responses to low frequency stimulation and species differences were noted. In addition, augmentation of the responses to high frequency stimulation was also seen in certain cases. This augmentation was not affected by the addition of atropine.

5. Physostigmine augmented the responses to fast and slow frequency stimulation in both rat and guinea-pig vas deferens. Unlike the action of the anti-adrenaline and local anaesthetic drugs, this effect was completely antagonized by atropine.

6. Neither anti-adrenaline drugs nor amphetamine had any ability to alter the pattern of action of the local anaesthetic drugs.

7. The ability to augment low frequency stimulation responses did not correlate with an ability to potentiate the effects of added noradrenaline on the vas deferens.

8. It is concluded that the ability to augment responses to low frequency stimulation does not involve either cholinergic mechanisms, nor a potentiation of the effects of the sympathetic transmitter.

It is a pleasure to acknowledge the technical assistance of Miss P. Russell. The samples of lignocaine and prilocaine were supplied by Astra Pharmaceuticals, to whom I am most grateful. Financial support was contributed by the National Heart Foundation of Australia to whom I express my thanks.

REFERENCES

ADLER, P., GAL, I. & VÉGH, L. (1950). Der Einfluss von Lokalanästhetica verschiedener Struktur auf die Cholinesterase-Aktivität im menschlichen Serum. *Zeitschr. fur Vitamin, Hormon, und Fermentforsch.* Vol. 3, p. 236–243.

BENTLEY, G. A. & SABINE, J. R. (1963). The effects of ganglion-blocking and post-ganglionic sympatholytic drugs on preparations of the guinea-pig vas deferens. *B.r J. Pharmac. Chemother.*, **21**, 190–201.

BENTLEY, G. A. (1965). Potentiation of responses to noradrenaline and reversal of sympathetic nerve blockade in the guinea-pig vas deferens. *Br. J. Pharmac. Chemother*, **25**, 243–256.

BOYD, H., CHANG, V. & RAND, M. J. (1960). The anticholinesterase activity of some antiadrenaline agents. *Br. J. Pharmac. Chemother.*, **15**, 525–531.

BÜLBRING, E. (1946). Observations on the isolated phrenic nerve diaphragm preparation of the rat. *Br. J. Pharmac. Chemother.*, **1**, 38–61.

BURN, J. H. & WEETMAN, D. F. (1963). The effect of eserine on the response of the vas deferens to hypogastric nerve stimulation. *Br. J. Pharmac. Chemother.*, **20**, 74–82.

DALE, H. H. & GADDUM, J. H. (1930). Reactions of denervated voluntary muscle and their bearings on the mode of action of parasympathetic and related nerves. *J. Physiol. (Lond.)*, **70**, 109–144.

DELALANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve blood vessel preparation. Aust. J. Exp. Biol. Med. Sci. **43**, 639–656.

DELLA BELLA, D., BENELLI, A. & GANDINI, A. (1964). Eserine and autonomic nervous control of guinea-pig vas deferens. *J. Pharm. Pharmac.* **16**, 779–787.

DELLA BELLA, D., GANDINI, A. & PRETI, M. (1964). The mechanism of the pressor responses to physostigmine in the rat and their modification by mebutamate and amylobarbitone. *Br. J. Pharmac. Chemother.*, **23**, 540–551.

DELLA BELLA, D., GANDINI, A. & PRETI, M. (1965). Influence of temperature on the responses of the guinea-pig hypogastric nerve-vas deferens preparation. *J. Pharm. Pharmac.*, **17**, 265–273.

FINKLEMAN, B. (1930). On the nature of inhibition in the intestine. *J. Physiol. (Lond.)*, **70**, 145–157.

HARVEY, A. M. (1939). The actions of procaine on neuromuscular transmission. *Bull. Johns Hopk. Hosp.*, **65**, 223–238.

HUKOVIĆ, S. (1961). Responses of the isolated sympathetic nerve-ductus deferens preparation of the guinea-pig. *Br. J. Pharmac. Chemother.*, **16**, 188–194.

MACCALLUM, M., MCCALLUM, J. A. N. & SHAW, F. H. (1949). The action of yohimbine on excitation and propagation in nerve. *Aust. J. exp. Biol. med. Sci.*, **27**, 115–122.

OHLIN, P. & STRÖMBLAD, B. C. R. (1963). Observations on the isolated vas deferens. *Br. J. Pharmac. Chemother.*, **20**, 299–306.

SHANES, A. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharmac. Rev.*, **10**, 59–274.