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EFFECTS OF COCAINE AND OF MONOAMINE OXIDASE AND CATECHOL-O-METHYL TRANSFERASE INHIBITORS ON TRANSMISSION TO THE GUINEA-PIG VAS DEFERENS

BY

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Both monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) are capable of metabolizing noradrenaline, and are associated with sympathetically innervated tissues (Blaschko, 1952 ; Axelrod, Albers & Clemente, 1959). However, neither of these enzymes shows a high substrate specificity towards noradrenaline, and attempts to attribute the inactivation of the sympathetic transmitter to enzymic breakdown have led to conflicting results. The current concept is that the major mechanism of inactivation is by uptake and binding of released noradrenaline in the nerve terminals (Kopin, 1964, 1966 ; Iversen, 1965). In addition a proportion of transmitter appears to be O-methylated by COMT (Axelrod, 1966). Although MAO may be active in deamination of previously O-methylated noradrenaline, the bulk of experimental evidence suggests that this is not a factor in termination of transmitter action. Rather MAO appears to be primarily concerned with the metabolism of noradrenaline within the nerve terminals (Kopin, 1964, 1966 ; Smith, 1966).

The musculature of the guinea-pig vas deferens contains a high level of noradrenaline (Falck, 1962), which is localized in nerve fibres (Sjöstrand, 1965), and the effects of drugs known to modify sympathetic transmission have confirmed that the excitatory nerves to this organ are predominantly noradrenergic (Huković, 1961 ; Bentley, 1962 ; Burnstock & Holman, 1962, 1964 ; Bentley & Sabine, 1963 ; Birmingham & Wilson, 1963). In accordance with the concept of noradrenaline inactivation being largely by neuronal binding, drugs which block the uptake of noradrenaline into nerve endings have been shown to potentiate the nerve-mediated contractions of the vas deferens (Huković, 1961 ; Cairncross, 1965). In addition, a recent study by Bhargava, Kar & Parmar (1965) showed that potentiation of the nerve-mediated contractions could be produced by inhibitors of both COMT and MAO. Moreover, potentiation was also produced in the presence of the O-methylated but not the deaminated product of noradrenaline. These results led Bhargava *et al.* (1965) to postulate that both COMT and MAO were concerned in the metabolism of nervously released noradrenaline, and that MAO was the enzyme most concerned in the actual inactivation process.

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In the present study an examination has been made of the effects of cocaine and of MAO and COMT inhibitors on the contractile response of the vas deferens to nerve stimulation and on the transmission process from nerves to single smooth muscle cells.

METHODS

The vas deferens of the guinea-pig was isolated together with the hypogastric nerve and pinned down on a Perspex block in a 10 ml. bath. Modified Krebs solution (Huković, 1961), aerated with 95% oxygen plus 5% carbon dioxide and maintained at 35.5–36° C, was run through the bath at a rate of approximately 2 ml./min.

Platinum ring electrodes embedded in Araldite were placed on the hypogastric nerve approximately 2 mm from its junction with the vas deferens for post-ganglionic stimulation. Square wave pulses of 2 msec duration were applied with a Grass S4 stimulator. Intracellular electrical events in the longitudinal musculature were recorded with capillary micro-electrodes filled with 2M-KCl and having a tip resistance of 20–80 MΩ. In experiments concerned with the time course of excitatory junction potentials, the voltage of nerve stimulation was adjusted so as to be just below that resulting in initiation of a propagated action potential. For measurement of resting membrane potential, the stimulation voltage was increased so as to cause an action potential to be fired following which the microelectrode was rapidly withdrawn from the recording cell in order to determine the extracellular potential. The criterion used for successful impalement of a cell was an action potential with an overshoot of at least 10 mV.

Contractile responses were examined using the isolated vas deferens mounted in a 50 ml. bath containing modified Krebs solution and maintained at 36° C. Post-ganglionic transmural stimulation was elicited with platinum ring electrodes placed around the proximal end of the vas deferens, and 10 sec bursts of square wave pulses of 2 msec duration and supramaximal voltage were delivered every 90 sec at alternate frequencies of 5 and 50 pulses/sec. The interval between stimulation periods was kept constant by means of an automatic timing device. Contractions were recorded with a frontal point writing lever on a smoked drum. In experiments concerned with the contractile effects of noradrenaline, the sensitivity of the muscle was increased by removal of the mesenteric investment (Bentley & Sabine, 1963).

Drugs used in the study were catechol, BDH; cocaine hydrochloride, BDH; noradrenaline bitartrate, Winthrop; pheniprazine (JB-516), Lakeside Laboratories; and tranylcypromine (Parnate), Smith, Kline & French.

In microelectrode studies drugs were added to the stock bottle of Krebs solution. In organ bath experiments the drugs were dissolved in distilled water and added to the bath in volumes of not more than 0.1 ml. All concentrations cited refer to the final concentration of salt.

Chronic reserpinization was performed by the administration of 5 mg/kg reserpine intraperitoneally 48 hr and 24 hr before removal of the vas deferens.

The statistical significance of differences was tested using Student's t test (Fisher, 1936) on the assumption that the difference was not significant.

RESULTS

Under the conditions of the present study, the mean resting potential of the smooth muscle cells of the longitudinal musculature was 59.5 mV (Table 1).

The pattern of successive excitatory junction potentials in response to low frequency hypogastric stimulation has been described by Burnstock & Holman (1961). At low frequencies of stimulation the membrane repolarizes to its resting level between successive excitatory junction potentials. At higher frequencies the depolarizations due to successive excitatory junction potentials are partially additive, resulting in a maintained depolarization of the membrane during the stimulating train (summation). In the present

investigation the threshold frequency of stimulation at which summation was observed varied between 1.5–2.0 pulses/sec in different preparations. However, this threshold remained constant in any one preparation for up to 6 hr.

Effects of cocaine

Cocaine (10^{-6} and 10^{-5} g/ml.) produced potentiation of the nerve-mediated contractions of the vas deferens in response to stimulation at 5 pulses/sec (Fig. 1). No prominent difference between the effects of these two concentrations of cocaine was noted. In the presence of cocaine, at both 10^{-6} and 10^{-5} g/ml., a potentiation of 5–30% was seen in 5 out of 9 experiments, a reduction of 20–50% in 2 and no effect in 1 experiment. In one additional experiment cocaine, 10^{-5} g/ml. produced a 100% potentiation. Cocaine 10^{-6} g/ml. had no, or a slight reducing, effect on the response to stimulation at 50 pulses/sec in 10 experiments, while cocaine 10^{-5} g/ml. caused a reduction of 10–60%. Washing out of the bath caused temporary potentiation of previously reduced responses to 5 pulses/sec in 2 out of 2 experiments and to 50 pulses/sec in 4 out of 10 experiments. In all remaining experiments washing merely restored the responses to both 5 and 50 pulses/sec to their control values.

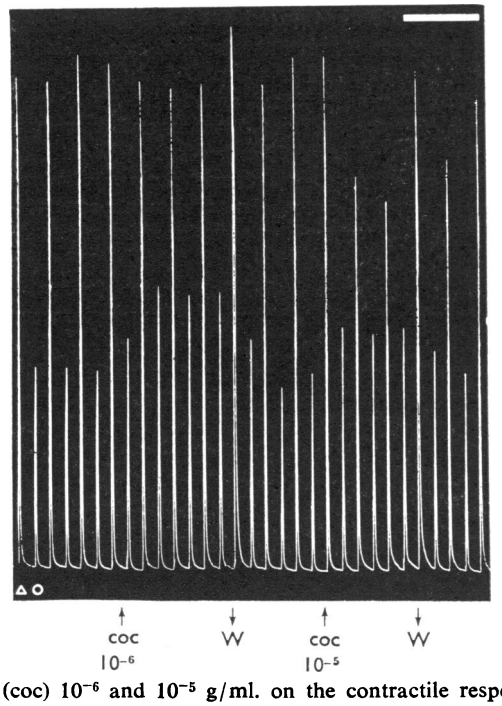

Fig. 1. Effect of cocaine (coc) 10^{-6} and 10^{-5} g/ml. on the contractile responses of the vas deferens to 10 sec periods of transmural stimulation every 90 sec at 5 (○) and 50 (△) pulses/sec alternately. W denotes washing. Time calibration: 10 min.

In contrast to its limited effects on the nerve-mediated response, cocaine markedly potentiated the response to applied noradrenaline (5×10^{-7}–10^{-6} g/ml.), both in amplitude and duration. This effect is illustrated by Fig. 2, which is a record obtained using the other vas deferens from the animal used to obtain Fig. 1.

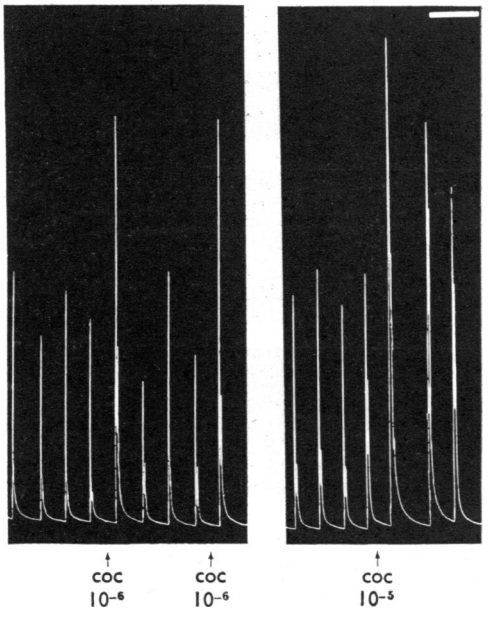

Fig. 2. The potentiating effect of cocaine (coc) 10^{-6} and 10^{-5} g/ml. on the responses of the stripped vas deferens to noradrenaline (10^{-6} g/ml. for 30 sec). This preparation was taken from the same animal as the one used to obtain Fig. 1. Time calibration: 10 min.

In 30 out of 34 cells impaled in 3 preparations, cocaine (10^{-6} g/ml.) caused summation of successive excitatory junction potentials during a train of stimulation at frequencies lower than those normally producing this effect (Fig. 3). This resulted in lowering of the voltage of nerve stimulation necessary to initiate a propagated action potential. In the remaining 4 cells impaled, no change in the time course of the excitatory junction potentials was observed. The time constants of decay of fully facilitated excitatory junction potentials in 4 preparations were calculated and found to be significantly increased by cocaine. Figure 4 compares the time course of the falling phase of typical excitatory junction potentials from 2 cells in the same preparation, before and after addition of 10^{-6} g cocaine/ml. In the presence of cocaine (10^{-5} g/ml.) progressive partial nerve block was produced, and frequently it was not possible to obtain excitatory junction potentials of appreciable size in response to low frequency stimulation. No observations have, therefore, been made of the effect of this concentration of cocaine on the excitatory junction potential time course. Both 10^{-6} and 10^{-5} g cocaine/ml. caused slight depolarization of the muscle membrane (Table 1).

Effect of MAO inhibition

As reported previously by Bhargava *et al.* (1965), the MAO inhibitors tranylcypromine and pheniprazine (2×10^{-6} g/ml.) caused potentiation of the nerve-mediated contractions of the isolated vas deferens (Fig. 5). These drugs also potentiated the contractile response to applied noradrenaline. The potentiation of nerve-mediated responses was not associated with any change in the time course of excitatory junction potentials. How-

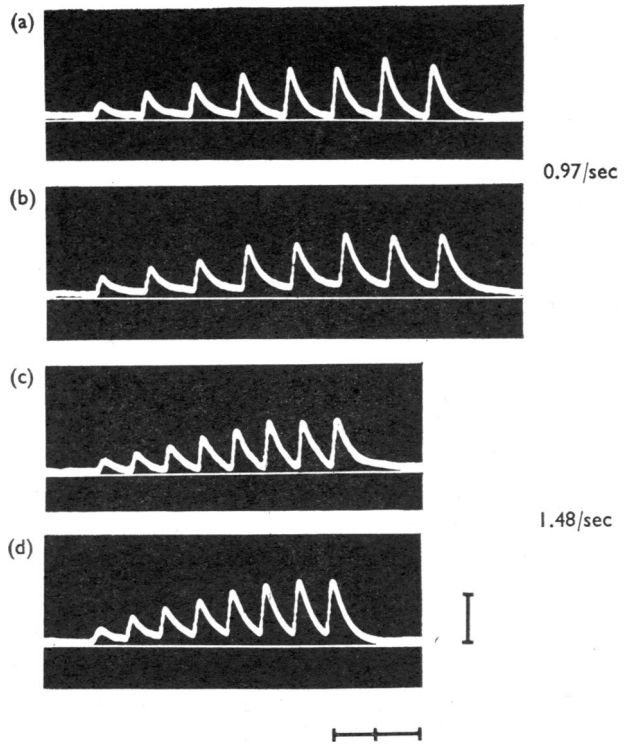

Fig. 3. Excitatory junction potentials recorded from smooth muscle cells of the vas deferens in response to post-ganglionic stimulation of the hypogastric nerve at 0.97 and 1.48 pulses/sec. (a) and (c) were recorded from a single cell under control conditions, and no summation was apparent (stimulation voltage 4.0 V). Cocaine (10^{-6} g/ml.) was then added to the bathing solution, and 30 min later (b) and (d) were recorded from a neighbouring cell. Summation was apparent at both frequencies of stimulation (stimulation voltage 3.5 V). Calibrations: 10 mV and 1 sec.

TABLE 1

EFFECTS OF COCAINE AND MAO AND COMT INHIBITORS ON THE MEMBRANE POTENTIAL OF SMOOTH MUSCLE CELLS IN THE VAS DEFERENS

Drug	Concn. (g/ml.)	Expts. (no.)	Cells (no.)	Membrane potential (mV)		Signif. of diff. from normal
				Range	Mean±S.E.	
	—	4	35	50–73	59·5±0·9	
Cocaine	10^{-6}	2	38	50–62	57·0±0·9	$P=0·05$
	10^{-5}	2	36	51–71	57·0±0·8	$P<0·05>0·02$
Tranylcypromine	2×10^{-6}	2	22	43–62	50·0±1·1	$P<0·01$
Pheniprazine	2×10^{-6}	2	33	41–65	54·0±1·0	$P<0·01$
Catechol	5×10^{-7}	3	50	45–70	56·5±1·1	$P<0·05>0·02$
	2×10^{-6}	2	30	48–67	54·0±1·0	$P<0·01$

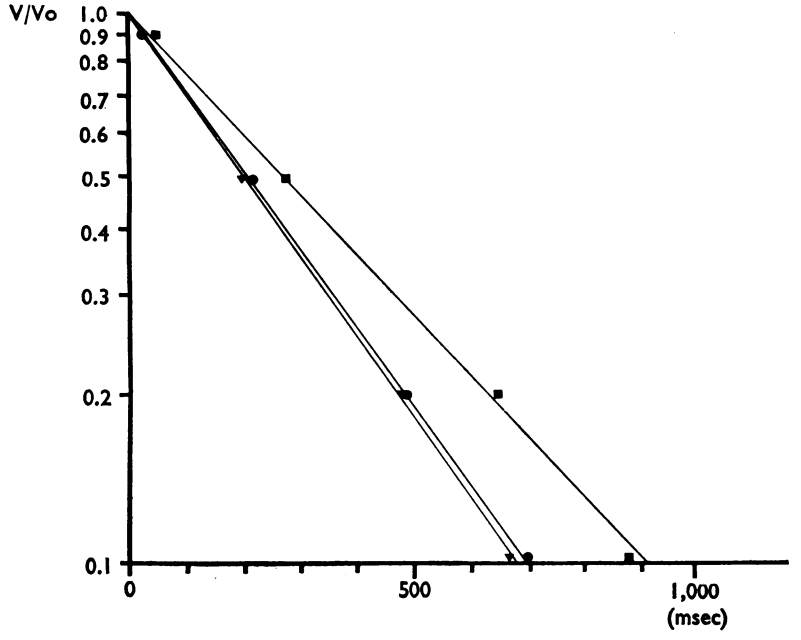

Fig. 4. Time course of the falling phase of 3 typical excitatory junction potentials (E. J. P's.) recorded during the course of a single experiment: (●) under control conditions (time constant of decay: 300 msec), (■) in the presence of cocaine 10^{-6} g/ml. (time constant of decay: 390 msec), (▼) after 60 min washing with normal Krebs solution (time constant of decay: 290 msec). Abcissa: proportion of amplitude of the E. J. P. at a given time (V) to maximal amplitude (Vo); Log scale. Ordinate: time after reaching maximal amplitude (msec).

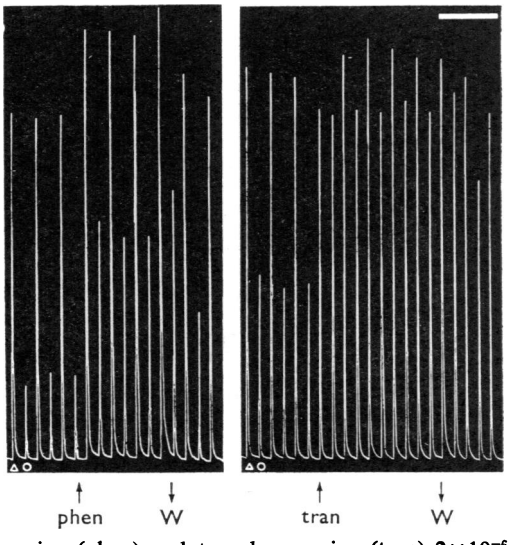

Fig. 5. Effects of pheniprazine (phen) and tranylcypromine (tran) 2×10^{-6} g/ml. on the contractile responses of the vas deferens to 10 sec periods of transmural stimulation every 90 sec at 5 (○) and 50 (△) pulses/sec alternately. W denotes washing. Time calibration: 10 min.

ever, the mean resting membrane potential of the muscle cells was lowered by 9.5 and 5.5 mV in the presence of tranylcypromine and pheniprazine respectively (Table 1), and the voltage of nerve stimulation for initiation of a propagated action potential was considerably reduced. In preparations taken from reserpinized animals no resting membrane depolarization (Table 2), nor reduction of stimulation voltage giving rise to

TABLE 2

EFFECTS OF COCAINE AND MAO AND COMT INHIBITORS ON THE MEMBRANE POTENTIAL OF SMOOTH MUSCLE CELLS IN THE VAS DEFERENS FOLLOWING PRETREATMENT OF THE ANIMAL WITH RESERPINE (5 MG/KG/DAY FOR 2 DAYS)

Drug	Concn. (g/ml.)	Expts. (no.)	Cells (no.)	Membrane potential (mV)		Signif. of diff. from normal
				Range	Mean ± S.E.	
(Reserpine alone)	—	4	43	47–71	58·0 ± 0·9	$P > 0{\cdot}1$
Tranylcypromine	2×10^{-6}	2	42	47–75	59·5 ± 1·3	$P > 0{\cdot}1$
Pheniprazine	2×10^{-6}	1	15	47–75	57·0 ± 1·8	$P > 0{\cdot}1$
Catechol	2×10^{-6}	2	29	46–77	58·0 ± 1·1	$P > 0{\cdot}1$

an action potential, was seen. Reserpinization also prevented potentiation of contractile responses to nerve stimulation by the MAO inhibitors (Fig. 6a). If, however, the nervous stores of noradrenaline were partially restored by incubation of the reserpinized tissue in noradrenaline (2×10^{-6} g/ml.) for 15 min, the MAO inhibitors then caused potentiation of the nerve-mediated contractions (Fig. 6b). Potentiation of the contractile response to applied noradrenaline was not abolished by reserpine treatment.

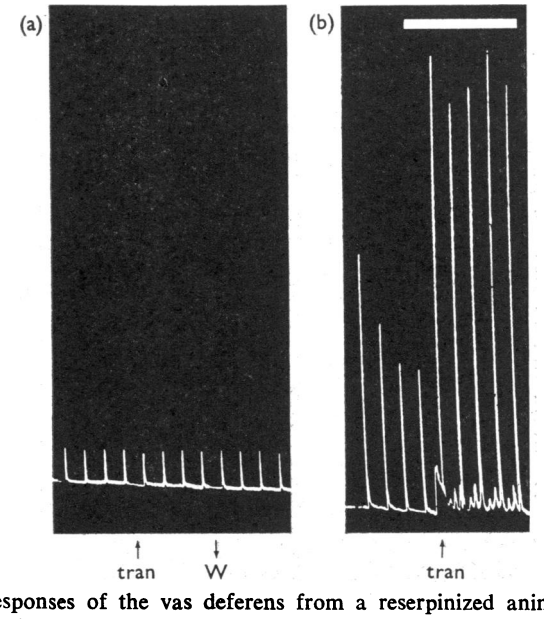

Fig. 6. Contractile responses of the vas deferens from a reserpinized animal to 10 sec periods of transmural stimulation every 90 sec at 10 pulses/sec. (a) lack of effect of tranylcypromine (tran) 2×10^{-6} g/ml. (b) following incubation of the tissue with 2×10^{-6} g/ml. noradrenaline for 15 min, showing enhancement of the responses to nervous stimulation and restoration of the potentiating effect of tranylcypromine. W denotes washing. Time calibration: 10 min.

Effect of COMT inhibition

Catechol, a potent inhibitor of COMT (Bacq, Gosselin, Dresse & Renson, 1959), weakly potentiated the contractions of the isolated vas deferens in response to low frequency stimulation at concentrations of 5×10^{-7}–2×10^{-6} g/ml. (Fig. 7). In 34 out of 40 cells impaled in 4 preparations, 2×10^{-6} g catechol/ml. caused summation of successive excitatory junction potentials during a stimulating train at frequencies lower than normal.

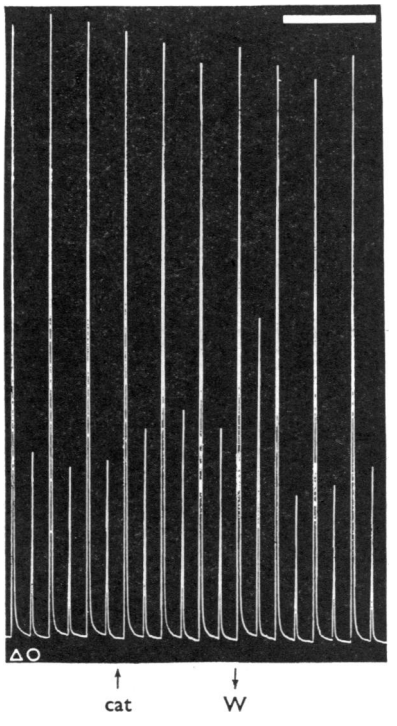

Fig. 7. Effect of catechol (cat) 2×10^{-6} g/ml. on the contractile responses of the vas deferens to 10 sec periods of transmural stimulation every 90 sec at 5 (○) and 50 (Δ) pulses/sec alternately. W denotes washing. Time calibration: 10 min.

Calculation of the time constants of decay of fully facilitated excitatory junction potentials in 4 preparations showed a significant increase in the presence of catechol (Table 3). In 2 of these preparations the increase was rather less than that seen in the presence of cocaine 10^{-6} g/ml. Figure 8 compares the time course of the falling phase of typical excitatory junction potentials from 2 cells in one of these preparations, before and after addition of 2×10^{-6} g catechol/ml. In 2 further preparations the prolongation of the excitatory junction potential in the presence of catechol was rather greater than that produced by cocaine (Table 3). No explanation can be offered for this variation. Although the voltage of nerve stimulation necessary to initiate a propagated action potential was sometimes transiently lowered soon after addition of catechol, partial failure of transmission consistently appeared within a short time, and in order to maintain

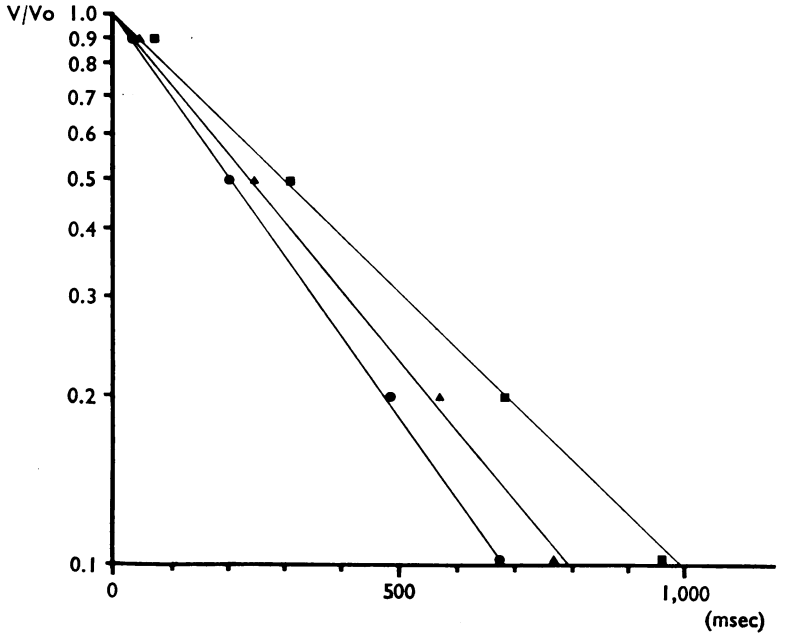

Fig. 8. Time course of the falling phase of 3 typical excitatory junction potentials (E. J. P's.) recorded during the course of a single experiment: (●) under control conditions (time constant of decay: 305 msec), (▲) in the presence of catechol 2×10^{-6} g/ml. (time constant of decay: 345 msec), and (■) after addition of cocaine 10^{-6} g/ml. in the presence of catechol 2×10^{-6} g/ml. (time constant of decay: 425 msec). Abcissa: proportion of amplitude of the E. J. P. at a given time (V) to maximal amplitude (Vo); log scale. Ordinate: time after reaching maximal amplitude (msec).

TABLE 3

TIME CONSTANTS OF DECAY OF FULLY FACILITATED EXCITATORY JUNCTION POTENTIALS UNDER CONTROL CONDITIONS AND IN THE PRESENCE OF COCAINE AND CATECHOL. SERIES (A) WAS PERFORMED IN DECEMBER 1966. SERIES (B) IN FEBRUARY 1967

Experimental series	Drug	Concn. (g/ml.)	Expts. (no.)	Cells (no.)	Time constant of decay (msec) Mean±S.E.	Signif. of diff. from control
(a)	—	—	4	30	280±10	—
	Cocaine	10^{-6}	2	20	360±17	$P<0{\cdot}001$
	Catechol	2×10^{-6}	2	20	440±16	$P<0{\cdot}001$
(b)	—		4	52	300±5	—
	Cocaine	10^{-6}	2	45	380±6	$P<0{\cdot}001$
	Catechol	2×10^{-6}	2	41	346±9	$P<0{\cdot}001$
	Cocaine (in presence of catechol)	10^{-6}	1	13	410±20	$P<0{\cdot}001$* $P=0{\cdot}05$†

* Refers to signif. of difference from value in presence of catechol alone.

† Refers to signif. of difference from value in presence of cocaine alone.

the size of excitatory junction potentials the voltage of nerve stimulation had to be raised above that used under control conditions. Despite the small degree of potentiation of nerve-mediated contractions, the mean resting membrane potential of the muscle cells was reduced in the presence of 2×10^{-6} g catechol/ml. by 5.5 mV (Table 1). This depolarization was not observed in preparations taken from animals which had received reserpine treatment (Table 2).

Effects of catechol plus cocaine

In 4 experiments performed, cocaine (10^{-6} g/ml.), added after maximal contractile potentiation had been obtained with catechol (2×10^{-6} g/ml.) caused further potentiation of the responses. This concentration of cocaine was added after catechol in one of the experiments reported in series (b) of Table 3, and was found to increase the time constant of decay of excitatory action potentials by a significant amount above that in the presence of either catechol or cocaine alone (Table 3, Fig. 8).

DISCUSSION

Cocaine potentiates the contractile responses of adrenergically innervated smooth muscle both to exogenous noradrenaline and to sympathetic nerve stimulation (Furchgott, 1955; Huković, 1959; Trendelenburg, 1959, 1963; Haefely, Hürlimann & Thoenen, 1964). It is generally accepted that this action is due to inhibition of inactivation of noradrenaline by neuronal uptake, and the evidence for this theory has been recently reviewed by Iversen (1965). Cocaine appears to cause a relatively specific blockade of catecholamine uptake, showing little MAO inhibiting activity (Brown & Hey, 1956), and no activity in inhibiting COMT (Holtz, Osswald & Stock, 1960). It may, therefore, be assumed that the effects of cocaine observed in the present study were attributable to its actions on tissue uptake of nervously released noradrenaline.

Cocaine potentiated the contractions of the vas deferens in response to low frequency nerve stimulation, and this was associated with prolongation of the excitatory junction potentials in the majority of muscle cells. These results are compatible with the theory that tissue uptake participates in removal of nervously released noradrenaline from the vicinity of its muscle receptors in the vas deferens.

Potentiation of the nerve-mediated response of the vas deferens by the MAO inhibitors tranylcypromine and pheniprazine, and the fact that the response could be augmented by addition to the bath of the O-methylated compound, normetadrenaline, but not by the deaminated metabolites of noradrenaline, led Bhargava *et al.* (1965) to postulate that deamination by MAO constitutes the main pathway of enzymic inactivation of noradrenaline in this tissue. The present study has confirmed that both tranylcypromine and pheniprazine potentiate the nerve-mediated response and shown that the response to applied noradrenaline is also potentiated. However, no prolongation of the excitatory junction potentials was observed in the presence of these drugs. Thus, no evidence has been obtained to support a role of MAO in the inactivation of nervously released noradrenaline. However, potentiation by the MAO inhibitors of the contractile responses to nerve stimulation and applied noradrenaline was associated with marked depolariza-

tion of the muscle membrane. Both this depolarization and the potentiation of nerve-mediated contractions were prevented by reserpinization. As no other effects of tranylcypromine or pheniprazine could be observed, it must be assumed that their potentiating action on contractile responses was due solely to their ability to depolarize the muscle membrane, and so facilitate the firing of action potentials.

The fact that the effects of the MAO inhibitors on both membrane potential and nerve-mediated contractions were abolished following reserpinization indicates that the depolarization was due to the action on the muscle membrane of noradrenaline from a neural source. This was supported by the fact that partial restoration of the nervous noradrenaline stores in reserpinized preparations restored the ability of the MAO inhibitors to potentiate nerve-mediated contractions.

Certain MAO inhibitors, including tranylcypromine, have been shown to inhibit neuronal uptake of noradrenaline (Iversen, 1965). However, cocaine, in concentrations which would have been expected completely to inhibit noradrenaline uptake, did not exert a marked effect on the membrane potential of the muscle cells. Thus, the depolarizing effect of the MAO inhibitors cannot readily be attributed to an effect on uptake. It has been postulated that, in the absence of normal intra-axonal metabolism by MAO, considerable amounts of noradrenaline may leak from the nerve terminals into the extracellular space (Triggle, 1965 ; Pletscher, 1966). The depolarization seen in the present study may have been due to such a situation. Alternatively, it is possible that pheniprazine and tranylcypromine are capable of actually releasing neuronal noradrenaline by a tyramine-like action. The actions of the MAO inhibitors are at present being more fully investigated.

Bhargava *et al.* (1965) reported that the COMT inhibitor, pyrogallol, potentiated the nerve-mediated response of the vas deferens, and tracer studies have shown that a considerable proportion of ^{3}H-noradrenaline released by stimulation of sympathetic nerves to the heart (Hertting, Axelrod, Kopin & Whitby, 1961), spleen (Hertting & Axelrod, 1961) and skeletal vascular bed (Rosell, Kopin & Axelrod, 1963) is O-methylated.

In the present study it was shown that the COMT inhibitor catechol weakly potentiated the contractile responses of the vas deferens to low frequency nerve stimulation, and that this was correlated with an increase in the time course of the excitatory junction potentials recorded in the majority of muscle cells. Metabolism by COMT, therefore, as well as neuronal re-uptake appears to participate in the inactivation of nervously released noradrenaline in the vas deferens.

In addition to causing prolongation of the excitatory junction potentials, catechol caused a lowering of the muscle membrane resting potential, which was prevented by prior reserpinization. This depolarization may be attributable to the accumulation in the vicinity of the muscle receptors of nervously-released noradrenaline which would normally have been metabolized. Similarly, the anticholinesterase physostigmine produces a depolarization of the muscle membrane, which is reversed by atropine, and which has been attributed to the persistence of nervously-released acetylcholine (Bell, 1967). It is notable that, although both catechol and pheniprazine caused a similar degree of depolarization of the muscle cell membrane, the potentiation of the nerve-mediated contractile response was considerably greater in the presence of pheniprazine than it was in the presence of catechol. Catechol, like pyrogallol, is a powerful oxygen capturer. It

is possible, therefore, that in the presence of catechol anoxic depression of the tissue occurred. Such a possibility is supported by the gradual failure of transmission seen during catechol treatment.

The pronounced potentiation by cocaine of contractile responses to applied noradrenaline indicates the importance of tissue uptake in the removal of noradrenaline from the extracellular space. However, neither cocaine nor catechol produced great prolongation of the excitatory junction potential. In comparison, inactivation of cholinesterase has been shown greatly to prolong the action of nervously released acetylcholine in the vas deferens (Bell, 1967). It may be that the termination of the action of nervously released noradrenaline in this tissue depends to a large extent on factors other than uptake and enzymic metabolism, such as diffusion.

SUMMARY

1. Intracellular recording from the smooth muscle cells of the longitudinal layer of the guinea-pig vas deferens has been used to examine the mechanism of inactivation of noradrenaline released by low frequency stimulation of the hypogastric nerve.

2. Cocaine (10^{-6} g/ml.) prolonged the excitatory junction potentials in response to post-ganglionic nerve stimulation and caused a slight lowering of the mean resting potential of the muscle cells. The contractile response to low frequency nerve stimulation was weakly potentiated by cocaine, while that to applied noradrenaline was markedly potentiated.

3. The monoamine oxidase (MAO) inhibitors tranylcypromine and pheniprazine (2×10^{-6} g/ml.) markedly potentiated the contractile response to nerve stimulation and applied noradrenaline, and lowered the mean resting potential of the muscle cells. No prolongation of the excitatory junction potentials was observed. The effects of the MAO inhibitors on membrane potential and the response to nerve stimulation were abolished by prior reserpinization.

4. The catechol-O-methyl transferase (COMT) inhibitor catechol (2×10^{-6} g/ml.) weakly potentiated the contractile response to low frequency nerve stimulation and prolonged the excitatory junction potentials. The mean resting potential of the muscle cells was lowered in the presence of catechol. This depolarization was prevented by reserpinization.

5. The effects of cocaine and catechol on both the nerve-mediated contractions and the time course of the excitatory junction potentials were additive.

6. It is concluded that noradrenaline released by low frequency nerve stimulation in the vas deferens is removed from the vicinity of the muscle receptors partly by uptake into the nerve terminals and metabolism by COMT. However, the relatively small effect of inhibition of both these pathways suggests that other factors are also involved. The potentiation of the nerve mediated contractile response of the vas deferens by MAO inhibitors appeared to be due to depolarization of the muscle membrane, probably by leakage of noradrenaline from the nerve terminals, and does not represent a role for MAO in metabolism of noradrenaline released by nerve stimulation.

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