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The effects of drugs on the sensitivity of the rat anococcygeus muscle to agonists

A. GIBSON AND D. POLLOCK

Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, Scotland

Summary

1. The effects of cocaine, 6-hydroxydopamine (6-OHDA), reserpine and 17-β-oestradiol on the sensitivity of the rat anococcygeus muscle to noradrenaline (NA), acetylcholine (ACh) and KCl were investigated.

2. Cocaine (10^{-5}M) increased the sensitivity of the anococcygeus to NA (100-fold) but not to ACh or KCl.

3. 6-OHDA treatment, whether discontinuous over 6 days or continuous for 12 days, also produced a specific increase in sensitivity to NA (50-fold).

4. Reserpine ((1 mg/kg)/day) produced a small increase in sensitivity of the muscle to NA and ACh but not to KCl, after 6 days and 12 days treatment.

5. 17-β-Oestradiol (10^{-5}M) had no effect on the sensitivity of the anococcygeus to NA or ACh, but reduced the sensitivity to KCl and the possible mechanism of this effect is discussed.

6. Cocaine increased, while reserpine, 6-OHDA and 17-β-oestradiol decreased, the maximum response of the muscle to KCl. The response to KCl was shown to consist of at least three components ; a direct action on the muscle, an effect due to release of NA, and an inhibitory action, probably due to release of the unknown inhibitory transmitter.

Introduction

In adrenergically-innervated tissues, supersensitivity of either surgical or pharmacological origin is generally thought to be of two distinct types (Trendelenburg, 1966). The first type is specific for catecholamines and is due to removal of the neuronal uptake process, so that the amount of amine reaching the biophase is increased. This type of supersensitivity is apparent within two days of denervation and is produced by cocaine or other drugs that block the catecholamine transport mechanism, characterized as Uptake$_1$ by Iversen (1967), and by 6-hydroxydopamine (6-OHDA), which destroys the adrenergic nerve endings.

The second type of supersensitivity is non-specific, is not restricted to directly acting sympathomimetic drugs but also occurs with unrelated agonists such as acetylcholine. This type, which may be post-synaptic in origin and appears after decentralization, denervation or reserpine treatment, generally requires five to seven days to develop. It occurs in a variety of tissues, including aortic strips (Hudgins & Fleming, 1966), the vas deferens (Birmingham, Paterson & Wojcicki, 1970) and the nictitating membrane of the spinal cat (Trendelenburg & Weiner, 1962). However, nonspecific supersensitivity does not occur in all tissues and is less likely to occur in isolated tissues. For example, it is absent in guinea-pig atria (Westfall &

Fleming, 1968), in the isolated nictitating membrane (Tsai, Denham & McGrath, 1968) and spleen (Green & Fleming, 1968) of the cat. There is no satisfactory explanation of the mechanism underlying non-specific supersensitivity. Neither is there a drug that can be relied upon to produce it. Even prolonged denervation does not necessarily produce non-specific supersensitivity in some tissues. These observations suggest that the ability of an effector cell to adapt to prolonged transmitter deprivation by becoming more sensitive to different agonists varies considerably in different tissues.

It was, therefore, of interest to establish whether the rat anococcygeus muscle, which has a dense adrenergic innervation, an unknown inhibitory transmitter and contracts to both noradrenaline (NA) and acetylcholine (ACh), (Gillespie, 1972), could develop both types of supersensitivity. The effects of two drugs, which generally produce different types of sensitivity, were investigated. These drugs were cocaine, which produces specific supersensitivity to NA, and reserpine, which produces non-specific supersensitivity in various adrenergically innervated tissues (Westfall, 1970). In addition, the effects of 17-β-oestradiol, which blocks extraneuronal catecholamine uptake (Iversen & Salt, 1970) were investigated. For comparison, the effects of 6-OHDA, which produces specific supersensitivity in this tissue (Gibson & Gillespie, 1973), were examined.

Methods

Male Wistar rats (150–250 g) were stunned and killed by exsanguination. The two anococcygeus muscles from each animal were dissected by the method described by Gillespie (1972). Each muscle was suspended in a 30 ml organ bath containing Krebs bicarbonate solution (mM: NaCl, 118·1 ; KCl, 4·7 ; $MgSO_4$, 1·0 ; KH_2PO_4, 1·2 ; $CaCl_2$, 2·5 ; $NaHCO_3$, 25·0 ; glucose, 11·1), which was maintained at 37° C and gassed with 95% O_2: 5% CO_2 throughout the experiment. The initial resting tension was adjusted to 0·2–0·5 g, and the responses to agonists were measured by means of Devices or Statham isometric transducers and a Devices M2 pen recorder.

All drugs, with the exception of KCl, were dissolved in 0·9% w/v NaCl solution (saline) and added to the bath in volumes not exceeding 0·4 ml. When KCl (1 M) was used to cause contractions, volumes of up to 5 ml were required to obtain the maximum response. In each muscle, dose-response curves were compiled for NA, ACh and KCl. The order in which the dose-response curves were obtained was randomized in order to avoid biasing the results. Responses were taken as the maximum increase in tension produced by each dose of the agonist. After each dose of agonist, subsequent doses were added only when the muscle tension had returned to its resting level.

Drugs or control solutions were either administered to the rats for several days prior to killing or were added to the bathing solution, so that their acute effects on the sensitivity of the tissue could be examined. Both 6-OHDA and reserpine were administered according to two dose schedules, the longer treatment being used in each case to allow sufficient time for the non-specific supersensitivity to develop. One group of rats received two doses (2×50 mg/kg, i.p.) of 6-OHDA on day 1 and a further two doses (2×100 mg/kg, i.p.) on day 4. These animals were killed on day 6, when the sensitivity of the anococcygeus muscles was examined. A second group of rats received 6-OHDA (50 mg/kg, i.p.) daily for eleven days. These rats were killed and the anococcygeus muscles examined on day 12. Reser-

pine (1 mg/kg, i.p.) was administered daily, either for 5 or 11 days, after which the rats were killed and the tissues examined on day 6 or 12 respectively.

In some experiments on tissues from untreated rats, cocaine was added to the Krebs solution to give a final concentration of 10^{-5}M. In other similar experiments, 17-β-oestradiol was added to the Krebs solution to give the same concentration but since it was only slightly soluble in water, it was first dissolved in 2 ml of ethanol, which was then dissolved in 3 litres of Krebs solution.

A regression line was calculated for each dose-response curve and from each line the pD_2 value (i.e. the $-\log_{10}$ of the dose of agonist which produces 50% of the maximum response) was calculated (Ariens & van Rossum, 1957). The mean pD_2 values for each agonist and treatment were calculated and compared by Student's t test.

The drugs used in this investigation were acetylcholine hydrochloride (Koch-Light), cocaine hydrochloride (Cockburn), guanethidine sulphate (Ciba), 6-hydroxydopamine hydrochloride (Calbiochem), (−)-noradrenaline bitartrate (Koch-Light), 17-β-oestradiol (Sigma), phentolamine mesylate (Ciba) and reserpine phosphate (Ciba).

Results

6-OHDA treatment, whether discontinuous over six days or continuous at a lower dose for twelve days, displaced the NA dose-response curve to the left (Figure 1). A 10-fold increase in sensitivity (Table 1) occurred without any alteration in the maximum response to NA. The discontinuous treatment with increasing doses of 6-OHDA produced a greater change in the sensitivity to NA than the lower dose, continuous treatment. 6-OHDA did not affect either the sensitivity of the anococcygeus to ACh and KCl or the maximum response to ACh (Fig. 1, Table 1). 6-OHDA reduced the magnitude of the maximum response to KCl. This observation suggested that part of the KCl-induced response may have been mediated by NA released from the adrenergic nerve endings by KCl. This possibility was confirmed by the use of the α-adrenoceptor antagonist phentolamine,

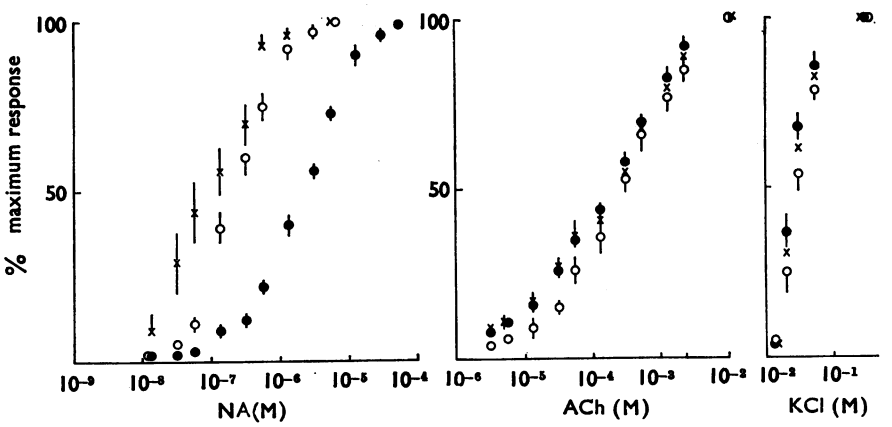

FIG. 1. Effects of 6-hydroxydopamine (6-OHDA) pretreatment on dose-response curves obtained with the anococcygeus muscle and different agonists, the concentrations of which are in molar units. ●=Control; ○=6-OHDA (12 days); ×=6-OHDA (6 days). Each point is the mean (±S.E.) of at least 6 observations.

TABLE 1. *The effects of drugs on the mean pD_2 values and maximum responses of the rat anococcygeus muscle to agonists*

	Noradrenaline			Acetylcholine			Potassium		
Treatment	Mean pD_2 values (±S.E.)	Maximum tension (g) mean (±S.E.)	*n*	Mean pD_2 values (±S.E.)	Maximum tension (g) mean (±S.E.)	*n*	Mean pD_2 values (±S.E.)	Maximum tension (g) mean (±S.E.)	*n*
Control	5·55±0·02	5·9±0·4	14	3·78±0·07	5·2±0·3	16	1·48±0·07	4·8±0·2	7
Cocaine (10^{-5}M)	7·58±0·19‡	6·5±0·5	7	3·92±0·11	5·3±0·4	8	1·50±0·02	6·7±0·6‡	6
6-OHDA									
(6 days)	7·09±0·24‡	6·9±0·7	7	3·75±0·11	5·9±0·5	8	1·45±0·05	3·8±0·4†	6
(12 days)	6·57±0·08‡	5·8±0·4	6	3·55±0·13	4·8±0·5	6	1·43±0·03	4·3±0·5	6
Reserpine									
(6 days)	5·80±0·10*	6·1±0·3	8	4·08±0·10†	5·4±0·3	11	1·54±0·03	3·6±0·3‡	11
(12 days)	5·79±0·11*	5·5±0·3	9	4·02±0·06†	5·5±0·3	10	1·47±0·03	3·2±0·4‡	6
17-β-Oestradiol									
(10^{-5}M)	5·55±0·06	6·1±0·3	8	3·64±0·13	4·6±0·3	8	1·05±0·03‡	3·1±0·3‡	7

Differences from the control value: * $0·05>P>0·01$; † $0·01>P>0·001$; ‡ $P<0·001$.

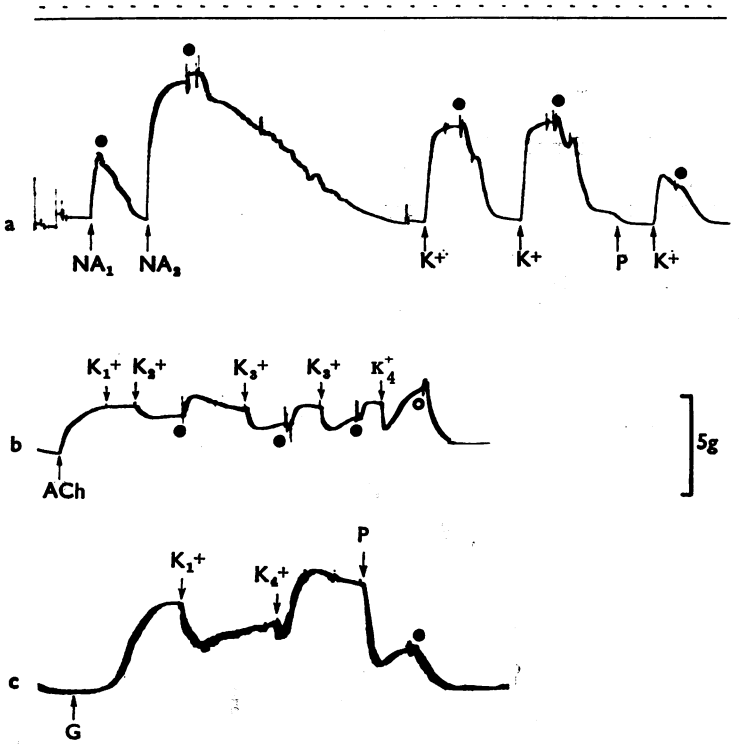

FIG. 2. (a) Responses of anococcygeus muscle from a control rat. At NA_1 and NA_2, 6×10^{-7}M and 10^{-4}M noradrenaline respectively were added to the bath; at K+ 6×10^{-2}M KCl and at P 6 μg/ml phentolamine were added. Phentolamine reduced the responses to KCl. ● denotes washout. Time scale: 1 min. (b) Responses of anococcygeus muscle from a rat pretreated with 6-hydroxydopamine ((50 mg/kg)/day for 11 days). Muscle tone was raised by acetylcholine (ACh, 3×10^{-4}M). At K_1+ (3×10^{-3}M), K_2+ (6×10^{-3}M), K_3+ (10^{-2}M) and K_4+ (3×10^{-2}M), KCl produced dose related inhibitions of the anococcygeus. ● denotes washout and replacement with Krebs+ACh (3×10^{-4}M). ○ denotes washout and replacement with Krebs. (c) Responses of anococcygeus muscle from a control rat. Tone was raised by 6×10^{-5}M guanethidine (G). At K_1+, 3×10^{-3}M and 3×10^{-2}M KCl respectively produced inhibition followed by contraction. Phentolamine (P) 6 μg/ml reduced the tone to that normally produced by KCl alone, while washout (●) reduced the tone completely.

which diminished the response to KCl (Figure 2a). Another possibility arising from these observations was that KCl might also release the unknown inhibitory transmitter (Gillespie, 1972) from nerve endings within the tissue. Evidence supporting this view was obtained with tissue from an animal treated with 6-OHDA. When the muscle tone was increased by ACh, KCl produced dose-dependent inhibitory responses, which were readily terminated by washing out the KCl (Figure 2b). In a normal anococcygeus muscle, maximally contracted by NA, KCl again produced inhibitions. When the tone of the muscle was raised by guanethidine, which acts as an indirect sympathomimetic in this tissue (Gillespie, 1972), the addition of a dose of KCl, normally sufficient to cause only a small contraction, now reduced the muscle tone. When a larger dose of KCl was used, there was a brief reduction in the muscle tone, followed by an increase in tension up to a level above that normally produced by that dose of KCl (Figure 2c).

Both short- and long-term treatment with reserpine produced spontaneous contractions and increased the rate of contraction and sensitivity of the anococcygeus to both NA and ACh (Fig. 3, Table 1). The maximum responses to these agonists were unaffected by reserpine pretreatment. In contrast, the sensitivity of the anococcygeus to KCl was unaltered but the maximum response to KCl was significantly reduced by reserpine (Table 1).

Cocaine also caused phasic contractions and produced a 100-fold increase in sensitivity to NA, without affecting either the sensitivity to ACh or to KCl, (Fig. 4, Table 1). Cocaine did not affect the maximum responses to NA or ACh but significantly increased the maximum KCl-induced response (Table 1), which was normally lower than the maximum response to NA.

17-β-Oestradiol did not affect either the sensitivity or the maximum responses of the anococcygeus to NA and ACh. It did, however, reduce both the sensitivity and maximum response of the tissue to KCl (Fig. 5, Table 1).

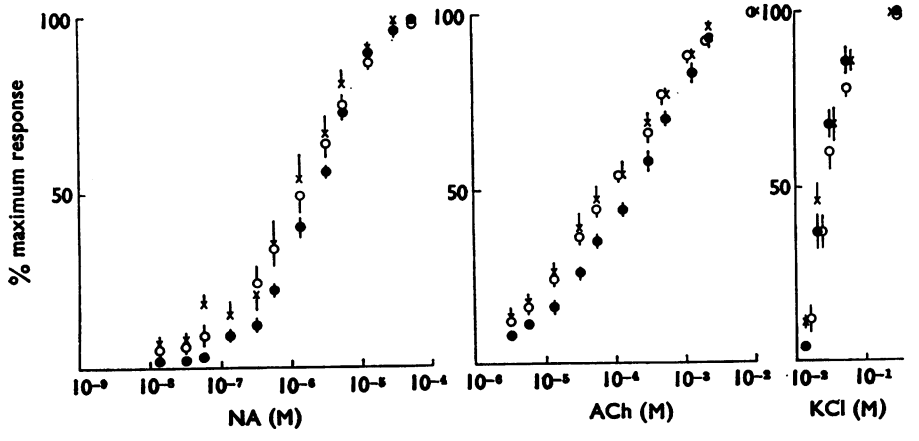

FIG. 3. Effects of reserpine pretreatment on dose-response curves obtained with the anococcygeus muscle and different agonists, the concentrations of which are in molar units. ●=Control; ○=reserpine (12 days); ×=reserpine (6 days). Each point is the mean (±S.E.) of at least 6 observations.

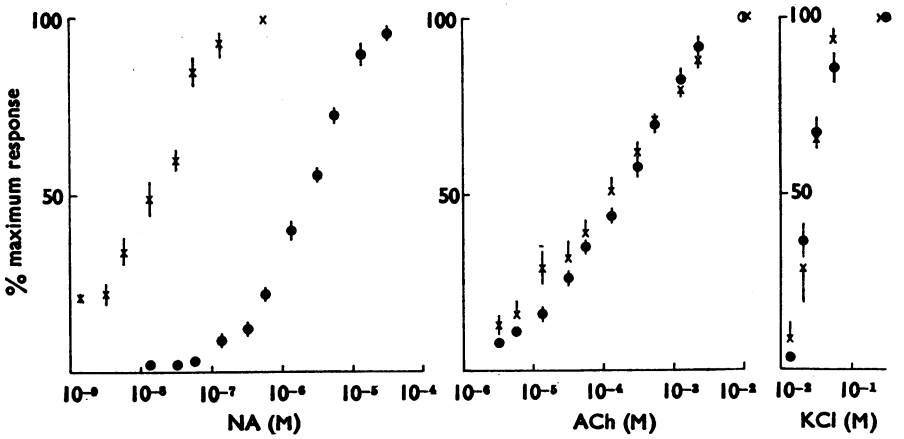

FIG. 4. Effects of cocaine (10^{-5}M) on dose-response curves obtained with the anococcygeus muscle and different agonists, the concentrations of which are in molar units. ●=Control; ×=cocaine. Each point is the mean (±S.E.) of at least 6 observations.

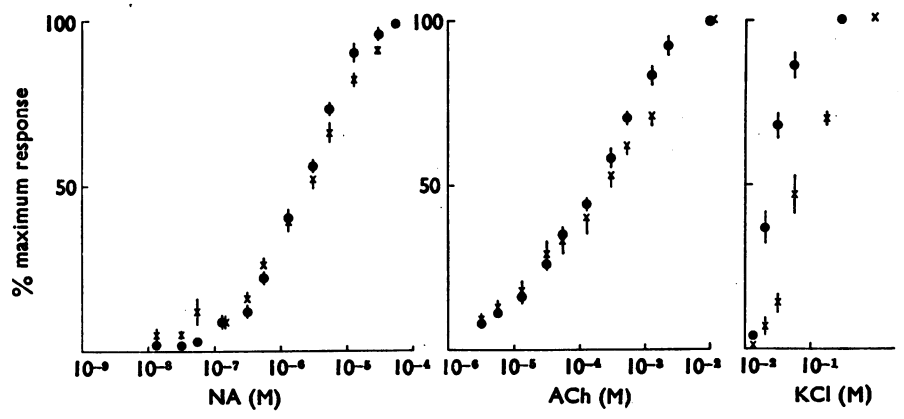

FIG. 5. Effects of 17-β-oestradiol (10^{-5}M) on dose-response curves obtained with the anococcygeus muscle and different agonists, the concentrations of which are in molar units. ●=Control; ×=17-β-oestradiol. Each point is the mean (±S.E.) of at least 7 observations.

Discussion

The specific increase produced by cocaine in the sensitivity of the anococcygeus to NA was consistent with the view that cocaine causes supersensitivity in adrenergically-innervated tissues by inhibiting the neuronal uptake mechanism (Trendelenburg, 1966). The similar but much less pronounced supersensitivity produced by 6-OHDA was comparable with that previously reported (Gibson & Gillespie, 1973) and was probably also due to the absence of the neuronal uptake process, in a tissue in which the sympathetic nerve endings had been destroyed. Clearly, the effects of 'chemical sympathectomy' (Bennet, Burnstock, Cobb & Malmfors, 1970) in the anococcygeus are unlike those produced by prolonged surgical denervation in the nictitating membrane (Trendelenburg & Weiner, 1962) in which non-specific supersensitivity occurs. In this study, even prolonged treatment with 6-OHDA did not produce non-specific supersensitivity. It is also interesting that 6-OHDA did not produce the same degree of supersensitivity to NA as

cocaine. It is possible that the full extent of the supersensitivity produced by 6-OHDA was partly masked by the adrenergic blocking activity of this drug (Haeusler, 1971). However, since this action is only seen at very high concentrations, a more likely explanation is that 6-OHDA did not produce a complete 'sympathectomy'. This view is supported by the observations that 6-OHDA does not completely abolish the motor response of the anococcygeus muscle to field stimulation (Gibson & Gillespie, 1973).

On the other hand, reserpine produced a slight but non-specific increase in the sensitivity of the anococcygeus to NA and ACh but not to KCl. Since the magnitude of the sensitivity increase was very similar for both ACh and NA, a similar mechanism may be involved. It is possible that reserpine produces this effect, directly or indirectly, by an action on the effector cell, perhaps at a stage beyond the receptors (Hudgins & Fleming, 1966). However, although the sensitivity of the anococcygeus to KCl was unaffected by either reserpine or 6-OHDA, both drugs reduced the magnitude of the maximum response to KCl and revealed something of the complexity of the action of KCl in this tissue, in which the normal response to KCl seems to be the resultant of at least three actions, a direct depolarizing action on the smooth muscle cell membrane and two indirect and opposing actions, mediated by NA and the unknown inhibitory transmitter, released respectively from adrenergic and inhibitory nerve fibres (Figure 2). These results suggest that drug-induced alterations in the sensitivity of a tissue to KCl must be interpreted with caution, especially in tissues such as the vas deferens, which is resistant to NA depletion by reserpine.

The inability of 17-β-oestradiol to affect the response of the anococcygeus to NA suggests that extraneuronal uptake of NA may not play a significant part in the disposal of NA in this tissue. On the other hand, the lack of any potentiation may merely reflect the fact that NA is a poor substrate for extraneuronal uptake, since in heart tissue hydrocortisone, which is a potent inhibitor of this uptake, does not potentiate the effects of NA (Kauman, 1972). Surprisingly, 17-β-oestradiol reduced both the sensitivity and maximum response of the anococcygeus muscle to KCl. A possible explanation of this observation is that 17-β-oestradiol increased the threshold of the nerve terminal membrane to depolarization by KCl, so that larger concentrations of KCl were required to release NA. If the steroid stabilized the nerve terminal membrane sufficiently, even the highest concentrations of KCl might be insufficient to release as much NA as in untreated tissues, so that the magnitude of the maximum response to KCl would also be reduced. Since neither the sensitivity nor the maximum response to NA or ACh was affected, it seems unlikely that 17-β-oestradiol affected the sensitivity of the postsynaptic membrane.

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