Br. J. Pharmac. (1977), **60**, 141–145

THE NATURE OF POTASSIUM CHLORIDE-INDUCED RELAXATIONS OF THE RAT ANOCOCCYGEUS MUSCLE

A. GIBSON & T.A. JAMES
Department of Pharmacology, Chelsea College, University of London, London SW3 6LX

1 The nature of KCl-induced relaxations of the rat anococcygeus muscle was investigated.

2 The relaxations were mimicked by other K^+ salts, but not by NaCl.

3 The muscle was more susceptible to the relaxant effects of KCl than the contractile effects.

4 Addition of ouabain (100 μM) had no effect on the relaxations.

5 The relaxations were abolished by tetrodotoxin (5 μg/ml), procaine (500 μM), and by section of the inhibitory nerves.

6 The results suggest that KCl-induced relaxations are due to stimulation of the inhibitory nerves by K^+.

Introduction

The actions of potassium chloride (KCl) on the isolated anococcygeus muscle of the rat are complex (Gibson & Pollock, 1973). On the resting muscle, which is devoid of tone, KCl produces dose-related contractions, which are due partly to direct depolarization of the muscle and partly to release of endogenous noradrenaline (NA). However, when the tone of the muscle is raised by acetylcholine (ACh) or guanethidine, KCl now produces dose-dependent relaxations.

At least two possibilities might explain these rather unusual relaxations of the muscle to KCl. Firstly, since KCl can cause contraction by releasing motor transmitter (NA), it was possible that the relaxations might be due to release of inhibitory transmitter thought to exist in this tissue (Gillespie, 1972). Secondly, it has been proposed that KCl might inhibit muscle activity by stimulating Na/K-adenosine triphosphatase (ATPase) activity in the cell membrane, thus causing hyperpolarization (Shibata, Fukuda, & Kurahashi, 1973; Johns & Paton, 1974).

The object of the present study was to characterize the nature of these KCl-induced relaxations of the anococcygeus muscle and to determine which, if either, of the above mechanisms might be involved.

A preliminary account of part of this work was given to the British Pharmacological Society (Gibson & James, 1976).

Methods

Male Wistar rats (200–300 g) were killed by stunning and exsanguination. The two anococcygeus muscles were then 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; and glucose 11.1) which was maintained at 37°C and gassed continuously with 95% O_2 and 5% CO_2. A resting tension of 0.5 g was placed on the muscle and changes in tension were detected by a Washington isometric transducer and recorded by a Washington 400 MD/2 pen recorder.

To evoke field stimulation the muscles were threaded through a pair of platinum electrodes embedded in Araldite. These were connected to a Palmer square wave pulse generator, which was used to stimulate the muscle at a frequency of 10 Hz, 1 ms, for 15 s, using a supramaximal voltage.

In order to produce relaxations to KCl, muscle tone was first raised by addition of ACh (40 μM). In most cases phentolamine (1 μM) was also added to offset any effect due to release of NA.

Denervation studies

Animals were anaesthetized with pentobarbitone (40 mg/kg). The anococcygeus muscles were approached via the scrotum, as outlined by Gillespie & McGrath (1973).

The object of these studies was to cut, if possible, the inhibitory fibres running to the anococcygeus muscle. However, since the exact neural pathways to the muscle have not yet been fully elucidated we could not be certain of the selectivity of our technique. Nevertheless it is thought that the inhibitory pathway enters the muscle on its ventral aspect, near the region where the two muscles unite. Nerve-like structures

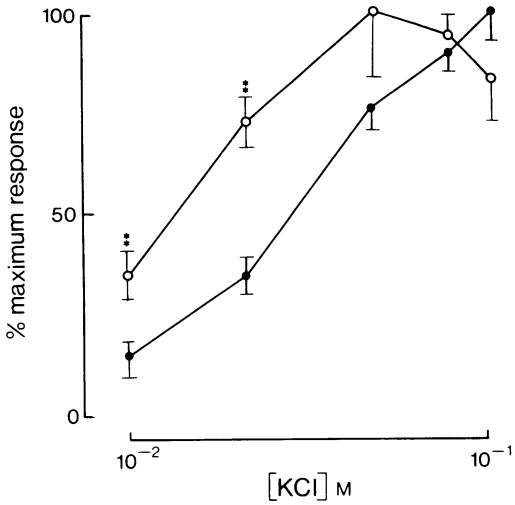

Figure 1 Dose-response curve of the contractile (●) and relaxant (○) effects of KCl on the rat anococcygeus muscle. Each point is the mean of at least six observations; vertical bars show s.e. mean. ** = $0.01 > P > 0.001$.

were sectioned in this area, and in addition the connective tissue between the colon and the anococcygeus was split. A further nerve pathway was seen to enter the muscle towards the spinal tendon. This nerve ran in close apposition to an artery and was left untouched.

One muscle in each animal was treated as above; the other was left untouched and served as a control.

Operated animals were then allowed 3 days to recover. On the 4th day the rats were killed, and the anococcygeus muscles dissected.

Drugs

The following drugs were used: acetylcholine chloride (Koch-Light), ouabain (Sigma), pentobarbitone sodium (Abbott), phentolamine mesylate (Ciba), procaine hydrochloride (Evans Medical) and tetrodotoxin (Sigma).

Results

Characteristics of relaxations

In order to compare the relative effectiveness of KCl in producing either contraction or relaxation of the anococcygeus muscle dose-response curves were obtained for each effect (Figure 1). These suggested that the muscle was more susceptible to the relaxant actions of KCl.

The relaxations produced by KCl were mimicked by other K^+ salts, including bicarbonate and tartrate (Figure 2). However, NaCl did not cause relaxation, but if anything produced a further increase of muscle tone.

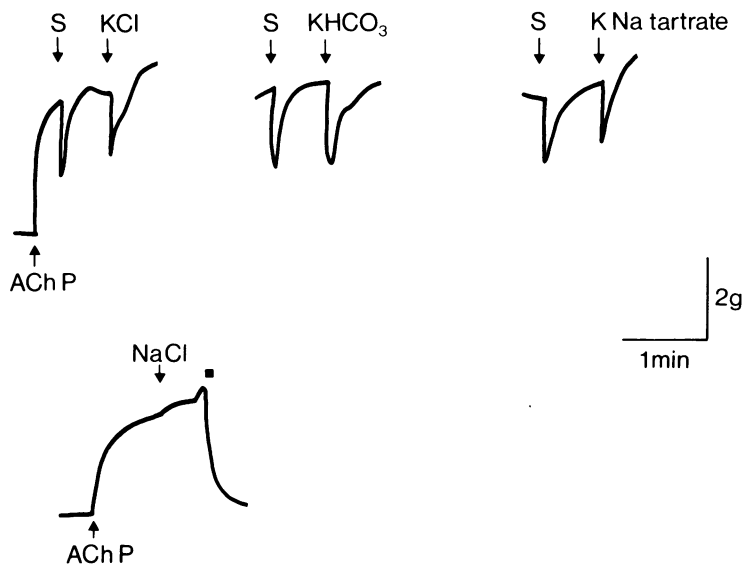

Figure 2 Effect of different salt solutions on the contracted anococcygeus muscle. S represents field stimulation at 10 Hz. At AChP, muscle tone was raised by acetylcholine (40 μM), in the presence of phentolamine (1 μM). All salts were added at a concentration of 20 mM.

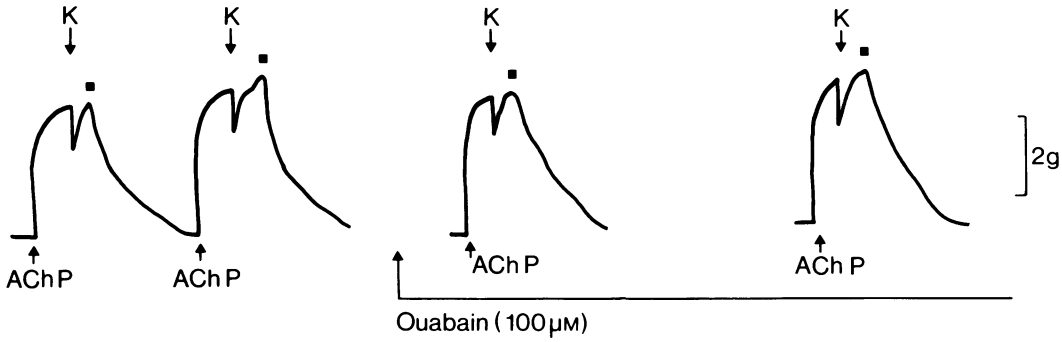

Figure 3 Effect of ouabain on KCl-induced relaxations of the anococcygeus muscle. At AChP muscle tone was raised by acetylcholine (40 μM) in the presence of phentolamine (1 μM). At K, KCl (20 mM) was added: ■ represents washout. The third and fourth traces were obtained 15 min and 30 min respectively after addition of ouabain (100 μM).

Effect of ouabain

Addition of ouabain (100 μM) to the bathing medium did not affect the relaxations produced by KCl, even after an incubation period of 30 min (Figure 3).

Effect of tetrodotoxin

Figure 4a shows the effect of tetrodotoxin (TTX) on KCl-induced relaxations of the muscle. TTX (5 μg/ml) produced a complete block of the relaxations, which returned when the TTX was washed out of the bath.

Effect of procaine

Before administration of procaine, the muscle was responding with relaxations to nerve stimulation and KCl (Figure 4b). However, procaine (500 μM) blocked

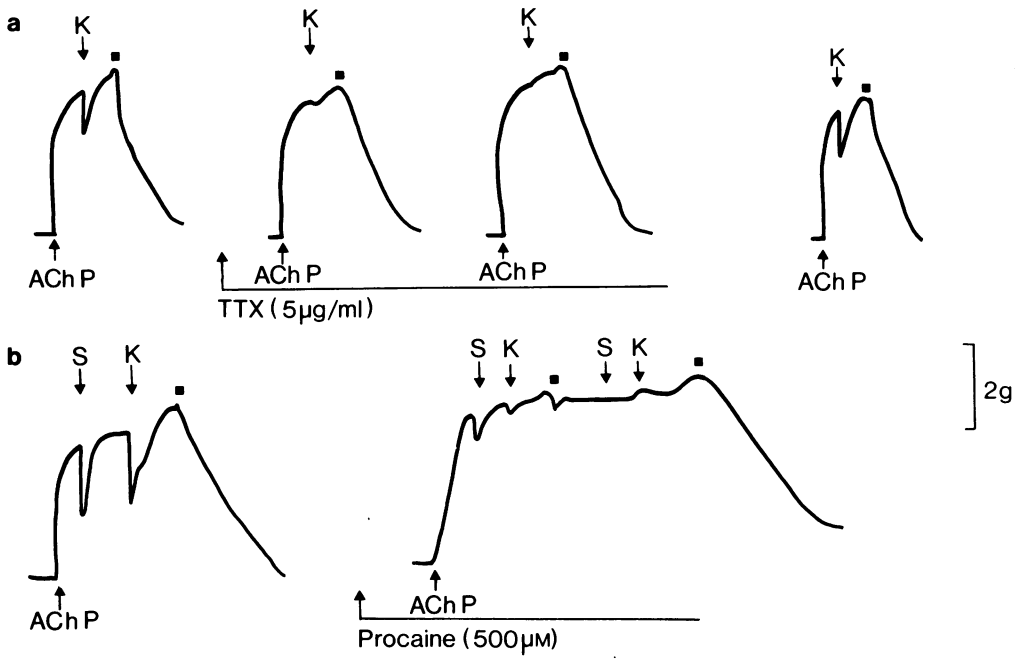

Figure 4 Effect of (a) tetrodotoxin and (b) procaine on KCl-induced relaxations. At AChP tone was raised with acetylcholine (40 μM) in the presence of phentolamine (1 μM). K represents addition of KCl (20 mM), S field stimulation (10 Hz, 1 ms, 15 s), and ■ washout.

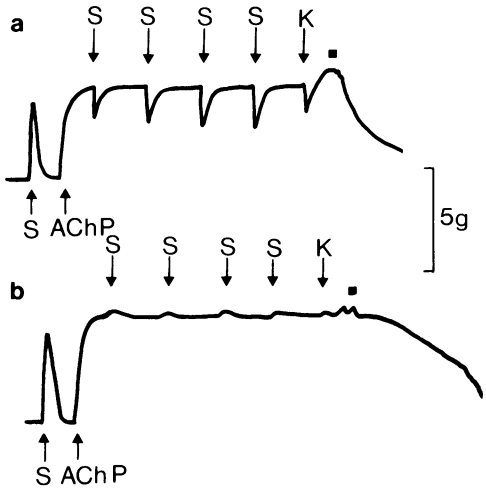

Figure 5 Effect of denervation on KCl-induced relaxations: (a) is from a control muscle; (b) from the contralateral denervated muscle. S represents field stimulation (10 Hz, 1 ms, 15s); AChP, addition of acetylcholine (40 μM) and phentolamine (1 μM); K addition of KCl (20 mM) and ■ washout.

the response to both procedures, although it had no effect on the background tone of the muscle nor on the relaxation following washout of ACh.

Effect of denervation

The contralateral control muscle responded to field stimulation with contraction (Figure 5a). When muscle tone was raised by ACh, field stimulation produced relaxations which were mimicked by KCl.

The 'denervated' muscle also responded to field stimulation with contraction (Figure 4b). This contraction could be abolished by phentolamine. In addition it was found that guanethidine (30 μM) retained its indirect sympathomimetic activity on the muscle. However, when muscle tone was raised by ACh, neither field stimulation nor KCl caused relaxations (Figure 4b).

Discussion

The main purpose of this study was to determine whether stimulation of Na/K ATPase or release of inhibitory transmitter might explain KCl-induced relaxations of the contracted anococcygeus muscle. The former possibility was tested using the Na/K ATPase inhibitor ouabain, which has been shown to prevent KCl-induced inhibitions of guinea-pig taenia coli (Shibata *et al.*, 1973) and of rabbit myometrium (Johns & Paton, 1974). However, in the rat anococcygeus muscle ouabain had no effect on the relaxations produced by KCl, although the concentration was 100 times that used in the previous studies. It would seem therefore that stimulation of Na/K ATPase activity is unlikely to explain the actions of KCl on the anococcygeus muscle.

The possibility that the release of an inhibitory transmitter explains KCl-induced relaxations was investigated in several ways. Firstly, tetrodotoxin, which has been shown to block the inhibitory response to field stimulation (Gillespie, 1972), completely blocked the KCl-induced relaxations. Secondly, the local anaesthetic procaine blocked both the response to field stimulation and to KCl. These results suggest that the relaxations to KCl are mediated through stimulation of a neural pathway, and therefore are probably due to release of the inhibitory transmitter.

However, to substantiate this probability further, the denervation studies were attempted. As stated previously, the exact details of the innervation pattern of the anococcygeus muscle is uncertain, and there is no histological method for identifying the inhibitory nerve terminals. Consequently, the only measure of a successful denervation was lack of relaxation to field stimulation, which was achieved in six out of seven experiments. In denervated muscles, addition of KCl to the bathing medium did not produce relaxations, supporting the theory that this effect of KCl is due to release of the inhibitory transmitter.

It is of interest that it is possible to destroy selectively the inhibitory fibres to the anococcygeus muscle, suggesting that there are two distinct pathways. The presence of the sympathetic nerves in denervated muscles was confirmed by contraction to nerve stimulation, and to the indirect sympathomimetic actions of guanethidine, both effects being antagonized by phentolamine. Thus, two of the criteria for the presence of adrenergic fibres in the anococcygeus muscle were satisfied (Gibson & Gillespie, 1973). The third criterion, demonstration of the presence of adrenergic fibres by fluorescence microscopy, was not attempted in this study.

It is possible that the sympathetic fibres are contained in the nerve seen to enter the muscle in close proximity to an artery, since this nerve was left untouched. Alternatively, McKirdy & Muir (1976) have suggested the presence of a ganglion in the motor pathway adjacent to the muscle, and thus it is possible that in the present experiments the sympathetic tract was cut preganglionically. In the case of the inhibitory nerves, it seems that the ganglion in this pathway (Gilliespie & McGrath, 1973) does not lie in close apposition to the muscle, since nerve section abolished responses to field stimulation.

Although chloride ions have been shown to be involved in both hyperpolarization and depolarization (Marshall, 1973; Constanti & Nistri, 1976) the active ion in KCl producing relaxation of the anococcygeus muscle is K^+, since the effect was mimicked by other

K^+ salts, but not by NaCl. Since the dose-response for the relaxant effects of KCl was to the left of that for the contractile effects, it would seem that the inhibitory nerves are more susceptible to depolarization by KCl than the motor nerves. In the case of field stimulation, the inhibitory nerves function at a lower frequency than the motor nerves (Gillespie, 1972; Gibson & Gillespie, 1973).

In conclusion, KCl-induced relaxations of the rat anococcygeus muscle appear to be due to stimulation of the inhibitory nerves by K^+, causing release of an unknown inhibitory transmitter.

References

CONSTANTI, A., & NISTRI, A., ((1976). A comparative study of the action of γ-aminobutyric acid and piperazine on the lobster muscle fibre and the frog spinal cord. *Br. J. Pharmac.*, **57**, 347–358.

GIBSON, A. & GILLESPIE, J.S. (1973). The effect of immunosympathectomy and of 6-hydroxydopamine on the responses of the rat anococcygeus muscle to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **47**, 261–267.

GIBSON, A. & JAMES, T.A. (1976). K^+-induced relaxations of the rat anococcygeus muscle. *Br. J. Pharmac.*, **58**, 305P.

GIBSON, A. & POLLOCK, D. (1973). The effect of drugs on the sensitivity of the rat anococcygeus muscle to agonists. *Br. J. Pharmac.*, **49**, 506–513.

GILLESPIE, J.S. (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **45**, 404–416.

GILLESPIE, J.S. & McGRATH, J.C. (1973). The spinal origin of the motor and inhibitory innervation of the rat anococcygeus muscle. *J. Physiol., Lond.*, **230**, 659–672.

JOHNS, A. & PATON, D.M. (1974). Inhibitory effect of sodium pumping on spontaneous contractility of rabbit myometrium. *Can. J. Physiol. Pharmac.*, **52**, 786–790.

MARSHALL, J.M. (1973). Effects of catecholamines on the smooth muscle of the female reproductive tract. *A. Rev. Pharmac.*, **13**, 19–32.

McKIRDY, H. & MUIR, T.C. (1976). The response of the rat anococcygeus muscle to stimulation of the individual extrinsic nerves and its modification by drugs. *Br. J. Pharmac.*, **57**, 434P.

SHIBATA, S., FUKUDA, H. & KURAHASHI, H. (1973). The inhibitory action of potassium on the mechanical and membranal activities of taenia from guinea-pig caecum. *Life Sci.*, **12**, 425–431.

(Received October 21, 1976.)