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CHARACTERIZATION OF THE ADRENOCEPTOR IN THE ISOLATED CREMASTER MUSCLE OF THE GUINEA-PIG

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1 Contractions of the isolated cremaster muscle of the guinea-pig obtained in response to direct electrical stimulation were augmented by salbutamol, isoprenaline, adrenaline, noradrenaline and orciprenaline (in that order of potency), and by potassium chloride. Phenylephrine and xylometazoline had no effect.

2 The augmenting effect of isoprenaline on the twitch response was not altered by yohimbine or tolazoline, but was totally prevented by butoxamine, (−) -1- (4-nitrophenyl)-2-isopropylaminoethanol (INPEA), (±) -INPEA, sotalol, metalol and practolol; (+) -INPEA was much less effective. The augmenting effect of potassium was not altered by β-adrenoceptor blockade.

3 The results are consistent with the view that the augmenting effect of the sympathomimetic agents is mediated through β_2- type of adrenoceptor in the skeletal muscle fibres.

Introduction

Catecholamines augment the tension and duration of maximal twitches of fast contracting mammalian skeletal muscle, but they have the opposite effect on slow contracting muscle (Goffart & Brown, 1947; Bowman & Zaimis, 1955; Bowman & Nott, 1969; Marsden & Meadows, 1970; Tashiro, 1973; Zaimis, 1973). There is substantial evidence that the effect of catecholamines on the slow skeletal muscle is mediated through the β_2-type of adrenoceptor (Bowman & Nott, 1970; Apperley, Daly & Levy, 1976). The directly evoked twitch responses of the guinea-pig cremaster muscle *in vitro* were increased by adrenaline (Kelkar, Gupta & Gokhale, 1976). In the present work an attempt is made to characterize the mechanism of this augmentation.

Methods

Cremaster muscle was obtained from freshly killed young animals (weighing 750 g or more). After displacing the testis into the abdominal cavity the cremaster was exposed as a cone shaped muscle with its apex attached to the epididymal pole of the testis, and the base fanning out towards the external abdominal ring (Kelkar *et al.*, 1976). The whole muscle was dissected out cutting across the muscle mass at the level of the external abdominal ring. The cone was slit open on two sides starting at the base and cutting upwards to points about 2 mm away from the apex. The strip so prepared was set up in an organ bath (20 ml) containing oxygenated Tyrode solution of the following composition (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, NaH_2PO_4 0.8, $NaHCO_3$ 12.5 and glucose 5.6, maintained at 37°C. A thin, coated platinum wire was used to attach the muscle to a spring loaded lever writing on a smoked kymograph paper. The wire and the platinum hook of the tissue holder served as two poles for direct muscle stimulation (submaximal single shocks, 20 ms, applied once every 20 seconds). The stimulation period (15 to 20 min) was followed by a rest period of 10 minutes.

The following drugs were used: (−) -adrenaline, salbutamol, and butoxamine (bases); orciprenaline sulphate; guanethidine sulphate; (+) -tubocurarine chloride; (±) -isoprenaline, isoxsuprine, (±) -noradrenaline, practolol, sotalol (MJ 1999), metalol (MJ 1998) (−) -1-(4-nitrophenyl) -2-isopropylaminoethanol (INPEA), (±) -INPEA, (+) -INPEA, (±) -*o*-hydroxy-3-(isopropylamino)-propoxy benzonitrile (Kö 1313), yohimbine, tolazoline, and verapamil were used as hydrochlorides. Concentrations are expressed as μM in the bath medium.

Results

The muscle responded reproducibly to direct stimulation, spontaneous variations in the twitch height

during individual 20 min stimulation periods being within 7% of the control ($n = 15$). Tubocurarine (14.37 μM, $n = 4$), verapamil (20.36 μM, $n = 5$) or guanethidine (3.37 μM, $n = 5$) did not alter the twitch height significantly.

Effect of agonists

The twitch responses were increased in the presence of salbutamol, isoprenaline, adrenaline, noradrenaline, and orciprenaline. The augmentation in the responses developed fully in 6 to 8 min and lasted for more than 45 min despite repeated washes. However, the effect was reversed by repeated washing after 5 min contact with a β-adrenoceptor blocking agent (Table 1). Tachyphylaxis was not observed to occur to any of the above named agonists.

Concentrations of the agonists producing a mean augmentation of 45 to 50% in the responses were: salbutamol, 10.9 μM ($n = 5$); isoprenaline, 14.23 μM ($n = 28$); adrenaline, 16.39 μM ($n = 11$); noradrenaline, 17.76 μM ($n = 6$); and orciprenaline, 38.4 μM ($n = 5$). The rank order of potency (isoprenaline = 1) was salbutamol (1.3) > isoprenaline (1.0) > adrenaline (0.87) $\geqslant$ noradrenaline (0.82) > orciprenaline (0.37). Ineffective agonists were phenylephrine (up to 70 μM, $n = 4$). xylometazoline (up to 60 μM, $n = 4$) and isoxsuprine (up to 5 μM, $n = 5$). Higher concentrations of isoxsuprine inhibited the twitch response (Table 1).

Addition of KCl to the bath to give 4 times the normal potassium concentration promptly resulted in a 100% or greater augmentation of the twitch responses. However, the effect disappeared after repeated washes and a rest period of 20 minutes. The augmenting effect of KCl was reproducible in 18 experiments, while in 5 experiments the effect was not satisfactory.

Effect of antagonists

In concentrations which had no inhibitory effect on the twitch response of the muscle, butoxamine, (−) -INPEA (Figure 1), (±) -INPEA, sotalol, metalol and practolol blocked the augmenting effect of isoprenaline on the twitch response; the minimal concentrations producing a nearly complete blockade of the isoprenaline response (Table 1), however, did not alter the augmenting effect of KCl. When lower concentrations of the blocking agents were used, the magnitude of blockade varied considerably in different experiments and satisfactory dose-effect relationships could not be established.

In concentrations which had no inhibitory effect on the twitch response Kö 1313 or (+) -INPEA afforded only a partial protection (Table 1) and isoxsuprine, yohimbine or tolazoline (Figure 1) afforded no protection against the augmenting effect of isoprenaline. Higher concentrations of (+) -INPEA or isoxsuprine (Table 1) markedly inhibited the twitch response; while isoxsuprine now afforded a considerable protection against isoprenaline, the protective effect of (+) -INPEA was no better than that of the lower concentration of this drug.

Table 1 Antagonism by drugs of isoprenaline-induced augmentation of the twitch responses of isolated cremaster muscle of guinea-pig to direct electrical stimulation

	Blockade of augmenting effect			*Reduction of established augmentation*	
Drug	*μM in bath*[a]	n	*Mean block (% control)*	*μM in bath*[b]	n
Butoxamine	11	4	100	33	4
(−)-INPEA	50	5	100	45	9
(±)-INPEA	50–75	4	100	45	8
Sotalol	65–80	4	100	110–160	3
Metalol	170–280	6	100	220–340	8
Practolol	200	5	100	280–800	6
(+)-INPEA	65	4	40	65	7
	250	3	40[c]	—	—
Kö 1313	3.7	5	55	4.8	9
Isoxsuprine	5	6	0	9.2	5
	8.5	4	75[c]	—	—
Yohimbine	8	5	0	—	—
Tolazoline	8	3	0	—	—

n, number of observations. [a]minimal effective concentrations; added to the bath 7 min before isoprenaline (14.23 μM) which produced a control 50% augmentation of twitch height. [b]this concentration produced a 90% reduction in the established effect of isoprenaline. [c]this concentration itself inhibited the twitch height by 30 to 40% of the control.

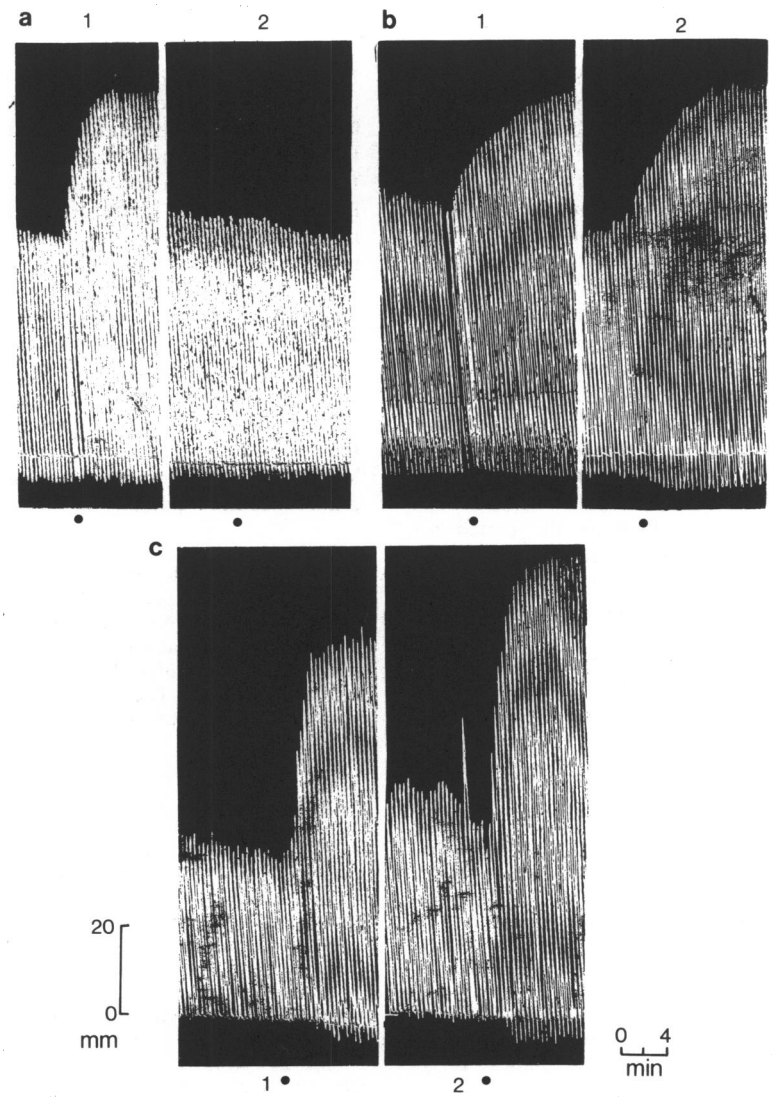

Figure 1 Isolated cremaster muscle of the guinea-pig. Direct electrical stimulation. Responses (at dots) to isoprenaline (14,23 μM, in a and b) and KCl (10.74 mM, in c). 1, control responses. 2, response 7 min after addition of (−)-INPEA (50 μM, in a and c) and of tolazoline (8 μM, in b).

In somewhat higher concentrations (Table 1) the β-adrenoceptor blocking agents and isoxsuprine reduced the previously established augmentation of the twitch response by 90%.

Discussion

The smooth muscle seen in the connective tissue on the vaginal surface of striated muscle in the tip of the guinea-pig cremaster muscle (Ninomiya, Merchant & Alonso deFlorida, 1976) actively participated in some responses of the muscle to pharmacological and electrical stimulation (Ninomiya, 1975). The present experiments used the whole cremaster muscle which did not contract in response to sympathomimetic drugs. It is therefore possible that the cremaster of animals in the higher weight range, such as those used in these experiments, have very little smooth muscle especially as the response of the muscle to

cholinergic drugs is blocked by tubocurarine (Kelkar *et al.*, 1976; Dale, Evinc & Vine, 1976; Dale & Muid, 1976). Furthermore, the twitch response in the present experiments was not inhibited in the presence of verapamil, a drug which was shown to abolish selectively the evoked slow contractions in the tip of guinea-pig cremaster, originating from the smooth muscle (Ninomiya, 1975). We assume therefore, that the twitch response to direct electrical stimulation in the present work was due to a contraction of skeletal muscle. The response did not seem to involve stimulation of voluntary or adrenergic nerves, since it was not influenced either by tubocurarine or guanethidine.

The responses of the cremaster muscle to direct stimulation were augmented not only by isoprenaline, adrenaline and noradrenaline, but also by the more specific β_2-adrenoceptor agonists, salbutamol (Cullum, Farmer, Jack & Levy, 1969) and orciprenaline (Engelhardt, Hoefke & Wick, 1961); the α-adrenoceptor agonists were without effect on the twitch response. Further, the augmenting effect of isoprenaline was blocked by several β-adrenoceptor blocking agents including the specific β_2-adrenoceptor antagonist, butoxamine.

Results obtained using stereoisomers of INPEA also indicate the involvement of β-adrenoceptors in the phenomenon, since, as in the case of trachea and heart (Patil, Miller & Trendelenburg, 1974), anti-isoprenaline activity of (+) -INPEA was weaker than that of (−) -INPEA. However, the exact potency ratio between the two could not be evaluated, since the former in high concentrations (Table 1) inhibited the twitch responses and the latter in lower concentrations produced a variable antagonism to isoprenaline. It seems unlikely that the β-adrenoceptor blocking agents were acting nonspecifically since many of them, viz., (−) -INPEA, (±) -INPEA, sotalol, metalol and practolol possess little membrane stabilizing activity. Furthermore, in concentrations which blocked the effect of isoprenaline, these drugs neither affected the electrical excitability of the tissue nor had any effect on the augmentation of the twitch response by KCl. The results suggest that the augmentation of the responses of the cremaster muscle to direct stimulation by sympathomimetic drugs is mediated by β-adrenoceptors, possibly of the β_2 type.

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References

APPERLEY, G.H., DALY, M.J. & LEVY, G.P. (1976). Selectivity of β-adrenoceptor agonists and antagonists on bronchial, skeletal, vascular and cardiac muscle in the anaesthetized cat. *Br. J. Pharmac.*, **57**, 235–246.

BOWMAN, W.C. & NOTT, M.W. (1969). Actions of sympathomimetic amines and their antagonists on skeletal muscle. *Pharmac. Rev.*, **21**, 27–72.

BOWMAN, W.C. & NOTT, M.W. (1970). Actions of some sympathomimetic bronchodilator and beta-adrenergic drugs on contractions of the cat soleus muscle. *Br. J. Pharmac.*, **38**, 37–49.

BOWMAN, W.C. & ZAIMIS, E. (1955). A comparison between responses of the tibialis anterior and the soleus muscles in the cat to adrenaline, noradrenaline and isoprenaline. *J. Physiol., Lond.*, **128**, 14–15.

CULLUM, V.A., FARMER, J.B., JACK, D. & LEVY, G.P. (1969). Salbutamol: a new selective β-adrenoceptive receptor stimulant. *Br. J. Pharmac.*, **35**, 141–151.

DALE, M.M., EVINC, A. & VINE, P. (1976). The denervated cremaster of the guinea-pig as a pharmacological preparation. *Br. J. Pharmac.*, **58**, 229–237.

DALE, M.M. & MUID, R. (1976). A simple *in vitro* preparation of mammalian tissue exhibiting properties of slow tonic skeletal muscle. *Br. J. Pharmac.*, **58**, 288–289.

ENGELHARDT, A., HOEFKE, W. & WICK, H. (1961). Pharmacology of sympathomimetic amine drug 1-(3,5-dihydroxyphenyl)-1-hydroxy-2-isopropylamino ethane. *Drug. Germ.*, **4**, 121–132.

GOFFART, M. & BROWN, G.L. (1947) Relation entre le potassium du milieu extracellulaire et l'action de l'adrenaline sue le muscle strie nonfatigue du rat. *C.r. Séanc. Soc. Biol.*, **141**, 958–959.

KELKAR, V.V., GUPTA, R.S. & GOKHALE, S.V. (1976). The isolated cremaster muscle preparation and (external) spermatic nerve-cremaster muscle preparation of the guinea-pig. *J. Pharm. Pharmac.*, **28**, 290–293.

MARSDEN, C.D. & MEADOWS, J.C. (1970). The effect of adrenaline on the contraction of human muscle. *J. Physiol.*, **207**, 429P.

NINOMIYA, J.G. (1975). Some pharmacological properties of the cremaster muscle of the guinea-pig. *Br. J. Pharmac.*, **55**, 487–495.

NINOMIYA, J.G., MERCHANT, H. & ALONSO DEFLORIDA, F. (1976). Ultrastructural arrangement and distribution of smooth muscle and striated muscle fibres in the cremaster of the guinea-pig. *Anat. Rec.*, **186**, 127–132.

PATIL, P.N., MILLER, D.D. & TRENDELENBURG, U. (1974). Molecular geometry and adrenergic drug activity. *Pharmac. Rev.*, **26**, 323–392.

TASHIRO, N. (1973). Effects of isoprenaline on contractions of directly stimulated fast and slow skeletal muscles of the guinea-pig. *Br. J. Pharmac.*, **48**, 121–131.

ZAIMIS, E. (1973). Spasticity alleviation by drugs acting directly on skeletal muscle. *Lancet*, **i**, 403–405.

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