One approach to the management of behavioural disorders with aggression is treatment with neuroleptics. Since their effective clinical value is restricted by side-effects (Peuch et al 1962), beclamide provides an approach attended by few side-effects and a wide margin of safety (Hawkes 1952).

A further advantage of the drug is that in acute doses (250 mg kg^{-1}) it causes no reduction of motor activity (Darmani et al 1987) and thus appears to produce its anti-aggressive action without major effects on other behaviours. Since drugs with anti-aggressive properties invariably induce an increase in 5-HT or a decrease in noradrenaline function, it may be hypothesized that beclamide produces its anti-aggressive effects, at least partially, through 5-HT release from presynaptic sites (Darmani et al 1986).

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The influence of urotensin II on calcium flux in rat aorta

A. GIBSON, S. CONYERS, H. A. BERN†, *Department of Pharmacology, Kings College London, Chelsea Campus, Manresa Road, London SW3 6LX UK, †Department of Zoology, University of California, Berkeley, California 94720, USA*

Abstract—The fish neuropeptide urotensin II (UII, 10 nM) caused a 51% increase in uptake of ^{45}Ca by segments of rat aorta; this increase was abolished by the Ca channel blocking drug nitrendipine (200 nM). ^{45}Ca efflux was unchanged in the presence of UII, but was significantly increased following washout of the peptide; again, this increase was not observed in the presence of nitrendipine. The results provide direct evidence that the nitrendipine-sensitive component of the contractile response of rat aorta to UII involves mobilization of extracellular Ca, with subsequent activation of a Ca-induced, Ca-release process intracellularly. The mechanisms responsible for the nitrendipine-resistant component of the contractile response to UII remain to be established.

Urotensin II (UII) is a dodecapeptide neurohormone which was first detected in the caudal neurosecretory system of teleost fish (Bern et al 1985). It has been known for some time that UII produces smooth muscle contraction and osmoregulatory changes in fish (Bern et al 1985), but it is only in recent years that clear biological effects have been demonstrated in mammalian species (Larson et al 1985). Of particular interest, in terms of pharmacology, has been the demonstration of potent effects of UII on cardiovascular function in rats (Gibson et al 1986). Most recently, two studies have shown that UII produces complex changes in tone of rat aorta in-vitro, causing endothelium-dependent relaxations (Gibson 1987) and more predominant, endothelium-independent contractions (Itoh et al 1987; Gibson 1987). Both studies reported preliminary investigations into the role of calcium ions (Ca) in the contractile response to UII. Gibson (1987) found that the contraction consisted of two components, phasic and tonic. The phasic response was dependent on extracellular Ca and was blocked by the Ca channel blocking drug nitrendipine; the tonic contraction was also dependent on extracellular Ca, but was more resistant to Ca depletion and was not blocked by nitrendipine. Itoh et al (1987) also found that a large component of the UII-induced contraction (about 50%) was inhibited by Ca channel blocking drugs, and that most of the residual response was dependent on extracellular Ca. However, these workers found a small component (about 10% of the original, full contraction) that persisted in the absence of extracellular Ca, and suggested that this might be due to Ca released from intracellular stores. Thus, the results

Correspondence to: A. Gibson, Department of Pharmacology, Kings College London, Chelsea Campus, Manresa Road, London SW3 6LX, UK.

from these two initial studies on contractile responses provided indirect evidence for the involvement of multiple Ca pools in UII-induced contraction, although there was some disagreement on the contribution of intracellular Ca. The aim of the experiments reported in the present study was to obtain more direct evidence for the role of different Ca pools in the contractile response of rat aorta to UII by determining the effect of the peptide on uptake and efflux of ^{45}Ca.

Methods

Male rats (Wistar strain, 200–400 g) were killed by stunning and bled from the neck. The thoracic aorta was cleared of surrounding tissue and excised from the aortic arch to the diaphragm. 6–10 mg segments were cut open to reveal the lumen and the endothelium removed by gentle rubbing with a cotton wool bud (Gibson 1987), moistened with physiological saline solution (PSS, see later). Each segment was then clamped in a small heart-clip, which was attached to a long wire handle, thus allowing easy, rapid transfer of the tissues between test tubes containing different media. The methods used to determine ^{45}Ca uptake and efflux were based on previously reported techniques (Godfraind 1976; Godfraind et al 1983; Dong & Wadsworth 1986).

^{45}Ca uptake. Tissue segments were equilibrated for 30 min in PSS (mM composition: NaCl 118·1, KCl 4·7, $MgSO_4$ 1·0, KH_2PO_4 1·2, $NaHCO_3$ 25·0, $CaCl_2$ 2·5, glucose 11·1, pH 7·4) which was gassed with 95% O_2:5% CO_2 and maintained at 37°C. They were then transferred to 2 mL PSS containing 0·5 μCi mL^{-1} ^{45}Ca (37°C) for 5 min to allow equilibration of the ^{45}Ca through the extracellular space, and then to 2 mL of the same solution (37°C) containing 10 nM UII or 200 nM nitrendipine or both, for a further 5 min. These concentrations of drug were those used in the original studies (Itoh et al 1987; Gibson 1987). Thereafter, the segments were washed for 30 min in 5 mL of ice-cold La^{3+} solution (mM composition: NaCl 118·1, KCl 4·7, $MgCl_2$ 1·0, $LaCl_3$ 50, glucose 11·1, Tris maleate 20, pH 6·9) to remove extracellular Ca from the tissue. Parallel control tissues, omitting the test drugs, were always carried through the procedure at the same time. After the La^{3+}-wash, each segment was blotted and weighed, and the accumulated ^{45}Ca was extracted overnight in 5 mM EDTA (1 mL). To this was then added 4 mL scintillation fluid, and the radioactivity in the sample counted in a liquid scintillation counter. Counting efficiency was estimated at 80% by the internal standard method, and the results were expressed as the apparent tissue content of ^{45}Ca (nmol ^{45}Ca g^{-1} wet weight tissue).

^{45}Ca efflux. Aortic segments were pre-incubated in PSS containing ^{45}Ca (2 μCi mL^{-1}) for 60 min at 37°C. Thereafter, they were sequentially transferred to test tubes containing 1 mL non-radioactive PSS (37°C) every 5 min for 110 min. The radioactivity released into each tube was counted after addition of 4 mL scintillation fluid. At the end of this period, ^{45}Ca remaining in the segment was extracted and estimated by the method described in the previous section. The rate coefficient of efflux, k (min^{-1}), was calculated from the equation:

$$k = \frac{^{45}Ca_{t1} - {}^{45}Ca_{t2}}{^{45}Ca_{t1} \times (t_2 - t_1)}$$

where $^{45}Ca_{t1}$ and $^{45}Ca_{t2}$ represent the ^{45}Ca content of the tissue at times t_1 min and t_2 min, respectively (Nayler & Sparrow 1983). Drugs used were: $^{45}CaCl_2$ (specific activity 23 mCi mg^{-1}, Radiochemical Centre, Amersham), nitrendipine (donated by Dr J. H. Botting, stock solution of 1 mM dissolved in absolute ethanol and diluted thereafter with 0·9% saline), urotensin II (synthetic *Gillichthys*, Peninsula, dissolved in distilled water). Statistical analysis was by Student's *t*-test.

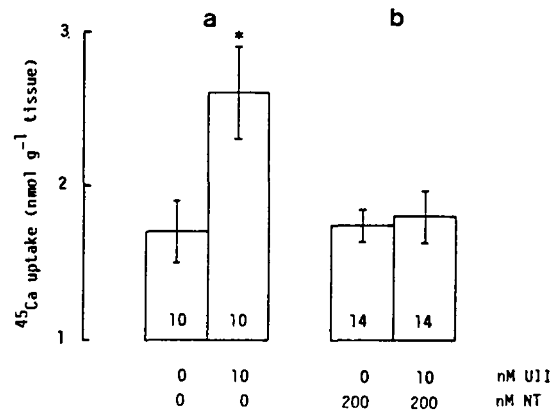

FIG. 1. Histogram showing the uptake of ^{45}Ca into segments of rat aorta, and the effect on this of urotensin II (UII) and nitrendipine (NT) acting either alone or in combination. The appropriate drug concentrations are given under each column, and the number of muscle segments studied in each group are given within the columns. Vertical bars represent s.e. of the mean. * $P<0·05$, value significantly different from uptake in the absence of any drugs.

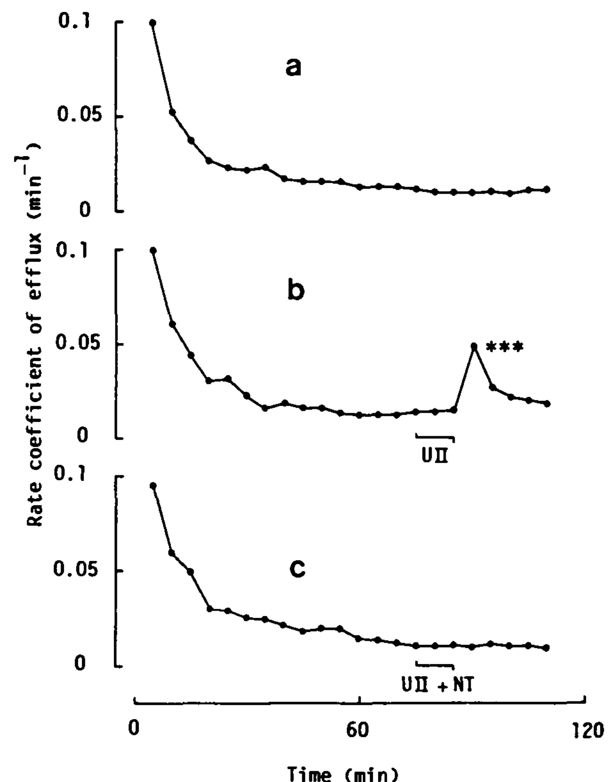

FIG. 2. Curves showing the rate of efflux of ^{45}Ca from segments of rat aorta at 5 min intervals after a 60 min pre-loading period. Each point is the mean from 6 muscle preparations; s.e. bars are not shown since they fall within the dimensions of the closed circles. The horizontal lines under the curves indicate the period of exposure to urotensin II (UII, 10 nM) and nitrendipine (NT, 200 nM). *** $P<0·001$, efflux significantly greater than immediate preceding value.

Results

The uptake of ^{45}Ca by rat aortic segments was significantly increased by 51% in the presence of 10 nM UII (Fig. 1a). Nitrendipine (200 nM) did not by itself affect ^{45}Ca uptake into the tissue, but it abolished the increase observed in the presence of UII (Fig. 1b).

In the absence of drugs, the rate of efflux of ^{45}Ca was rapid at

first, but slowed progressively to reach a nearly steady state by 60 min (Fig. 2a). The effect of UII on efflux was therefore estimated by inclusion of 10 nM UII in the two tubes between 75 and 85 min in the efflux curve. In the presence of UII, there was no change in the rate of ^{45}Ca efflux (Fig. 2b). However, the efflux rate was significantly increased following washout of the peptide (Fig. 2b). In the presence of 200 nM nitrendipine, UII by itself again did not affect efflux, but in this case there was no increase on washout of the drugs (Fig. 2c).

Discussion

The observation that 10 nM UII caused a 51% increase in uptake of ^{45}Ca confirms that the fish neuropeptide produces potent biological changes in rat aorta. It also provides direct evidence that at least part of the contractile response to UII involves mobilization of extracellular Ca (Itoh et al 1987; Gibson 1987). The ^{45}Ca uptake observed is probably that associated with the phasic component of the contraction since it was totally blocked by nitrendipine, which has already been shown to abolish selectively the phasic contractile responses (Gibson 1987). All the experiments carried out in this study utilized endothelium-denuded preparations, as UII may release relaxant factors from aortic endothelial cells (Gibson 1987); Ca mobilization into or out of endothelial cells as a result of this action may complicate results if the endothelium is not removed.

Previous experiments on aorta have shown increased ^{45}Ca efflux in the presence of some contractile agents, such as prostaglandin $F_{2\alpha}$ (Godfraind et al 1983), noradrenaline (Godfraind et al 1982; Dong & Wadsworth 1986), and phenylephrine (Godfraind et al 1982), but not in the presence of others such as clonidine (Godfraind et al 1982). Increased ^{45}Ca efflux induced by an agonist suggests that mobilization of intracellular Ca is an important component of the contractile response. In this study, ^{45}Ca efflux was unchanged in the presence of UII but was clearly increased in the period coinciding with muscle relaxation after washout of UII (Gibson 1987). This increased efflux suggests that exposure of the aorta to UII did produce an intracellular release of Ca; the delay in observing the increase might be explained by the proposal of van Breemen et al (1980) that the bulk of ^{45}Ca released intracellularly is not immediately eliminated from cells, but is sequestered into some other site from which it is subsequently released and extruded from the cell during relaxation (Droogmans et al 1985). The increased efflux was, however, abolished by nitrendipine indicating that it was dependent upon prior movement of Ca into the cell; consequently, the increased efflux might result from activation of a Ca-induced Ca-release process within the cell (Itoh et al 1985). The results of the present study strongly suggest that the nitrendipine-sensitive, phasic component of the contractile response to UII results from entry of Ca through voltage dependent Ca channels with resultant activation of Ca-induced Ca-release intracellularly.

One problem that remains to be solved is the nature of the nitrendipine-resistant, tonic component of the contractile response to UII, since no change in ^{45}Ca uptake or efflux was observed in the presence of nitrendipine. Organ bath studies established that the total contractile response to UII is Ca-dependent (Gibson 1987) but the present results give no indication of the pool of Ca responsible for the tonic contraction. One possibility is that the appropriate Ca pool may not be labelled by the methods used; for example, it may be the poorly-exchangeable Ca pool bound to the external surface of the plasma membrane (van Breemen et al 1982). Another possibility is that the amount of Ca mobilized may be too small to be detected; this would require concomitant activation of a Ca-amplification system by UII to generate contraction, probably via a second messenger. Adenylate cyclase appears to be ruled out since Itoh et al (1987) found no change in cAMP levels in aorta in the presence of UII. However, indirect evidence has been obtained for a possible activation of phospholipase C by UII, since aortic contractions were blocked by mepacrine, *p*-bromophenacyl bromide (Gibson 1987), and the more selective (Streb et al 1985) phospholipase C inhibitor neomycin (5 mM, Gibson unpublished observation). Phospholipase C activation results in the production of several second messengers, such as inositol trisphosphate, inositol tetrakisphosphate, and diacylglycerol (Michell 1986). Diacylglycerol activates protein kinase C, which is known to enhance greatly cellular sensitivity to Ca in several systems, even to the extent of causing activation of Ca-dependent processes in the absence of any rise in cytosolic Ca concentrations (Baker 1984). Clearly, a full understanding of the mechanisms underlying the contractile response of rat aorta to UII will require knowledge of the second messenger systems activated on interaction of UII with its receptors.

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