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Prejunctional modulatory action of neuropeptide Y on peripheral terminals of capsaicin-sensitive sensory nerves

[1]Sandro Giuliani, Carlo Alberto Maggi & Alberto Meli

Pharmacology Department, Research Laboratories, A. Menarini Pharmaceuticals, Via Sette Santi 3, 50131 Florence, Italy

1 We have determined the effect of neuropeptide Y (NPY) on motor responses produced by activation of capsaicin-sensitive primary afferents in the guinea-pig isolated left atria (reserpine-pretreatment, atropine in the bath) and bronchi (atropine and indomethacin in the bath) using capsaicin itself and electrical field stimulation as stimuli.

2 In both preparations, NPY inhibited or suppressed the response produced by electrical field stimulation while leaving the response to a submaximal concentration of capsaicin unaffected.

3 NPY had no effect on motor responses produced by a submaximal concentration of calcitonin gene-related peptide (atria) or neurokinin A (bronchi), the putative endogenous mediators of the responses produced by activation of the 'efferent' function of sensory fibres in these preparations.

4 We conclude that NPY exerts a prejunctional inhibitory action on transmitter release from peripheral endings of capsaicin-sensitive nerves. Failure of NPY to modulate responses activated by capsaicin provides further evidence for the existence of two independent modes of activation of the 'efferent' function of capsaicin-sensitive sensory nerves.

Introduction

Capsaicin-sensitive primary sensory neurones synthesize several neuropeptides (tachykinins, calcitonin gene-related peptide, CGRP) which are transported to both central and peripheral nerve endings at which level the peptides are released to produce their sensory and 'efferent' function, respectively (Szolcsányi, 1984; Maggi & Meli, 1988; Holzer, 1988). Pharmacological evidence indicates that these sensory nerve endings are endowed with various receptors for transmitters and autacoids (opioids, γ-aminobutyric acid (GABA), prostaglandins etc.), the activation of which produces changes in excitability, thereby regulating the amount of released transmitter (Yaksh *et al.*, 1980; Go & Yaksh, 1987; Barthó *et al.*, 1987; Saria *et al.*, 1988a,b; Maggi *et al.*, 1988a,b; 1989a; Giuliani *et al.*, 1989). These modulatory influences can be also observed in peripheral tissues indicating that the 'efferent' function of these primary sensory neurones could be regulated by other components of the autonomic nervous system. For instance, nicotinic and $GABA_A$ excitatory receptors and inhibitory α_2-adrenoceptors modulate excitability of capsaicin-sensitive afferents in the periphery (Saria *et al.*, 1988a,b; Maggi *et al.*, 1989a). Neuropeptide Y (NPY) is a 36 amino acid peptide, widely distributed in the mammalian peripheral nervous system, which has been shown to be present in sympathetic nerves where it is co-localized with noradrenaline and released by electrical nerve stimulation (Lundberg *et al.*, 1982; 1983; 1984; Fried *et al.*, 1985; Stjarne *et al.*, 1986). A dense plexus of nerve fibres containing NPY-like immunoreactivity is present in the heart and respiratory tract of various species (Sternini & Brecha, 1985; Lundberg *et al.*, 1983; Uddman *et al.*, 1984; Sheppard *et al.*, 1984; Potter, 1988). Among other effects, NPY has been shown to exert a prejunctional inhibitory influence on transmitter release from both cholinergic and noradrenergic nerves in the guinea-pig heart (Lundberg *et al.*, 1984; Kilborn *et al.*, 1985; Potter, 1987).

Here we present data indicating that NPY exerts a potent inhibitory action on motor responses produced in the guinea-pig atria and bronchi when

[1] Author for correspondence.

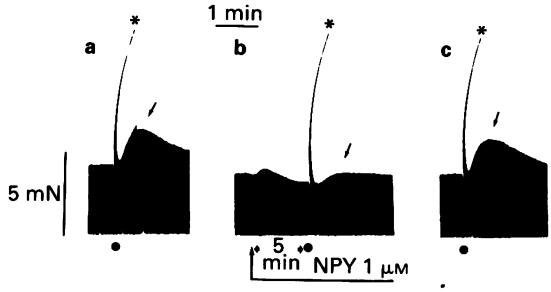

Figure 1 Typical tracings showing the early myogenic potentiating effect (indicated at asterisks) the delayed positive inotropic response (indicated at arrows) to electrical train field stimulation (applied at ●) of the guinea-pig isolated left atria (reserpine pretreated, atropine in the bath) which is due to antidromic activation of capsaicin-sensitive sensory nerves. In (b) neuropeptide Y (NPY, 1 μM) produced transient positive and negative inotropic effects and inhibited the delayed inotropic response to field stimulation. In (c) recovery from the inhibitory effect of NPY after 30 min thorough washing.

these sensory fibres are activated by electrical field stimulation but not by capsaicin. In view of the particular interest in interactions between sympathetic and sensory nerves in the mechanisms of pain generation (Wall & Gutnick, 1974; Levine *et al.*, 1986) the present data provide a further link whereby sympathetic nerves could influence excitability of sensory nerves, at least with regard to their 'efferent' function.

Methods

Guinea-pig atria

Inotropic responses of electrically driven guinea-pig left atria due to antidromic activation of capsaicin-sensitive nerves were obtained in a preparation similar to that described previously (Maggi *et al.* 1988b, 1989a; Giuliani *et al.*, 1989). Male albino guinea-pigs weighing 280–350 g received intraperitoneal reserpine (5 mg kg^{-1}) 48–96 h beforehand and were stunned and bled. The whole heart was rapidly removed and placed in Tyrode solution containing atropine (1 μM). The left atrium was cleaned from adhering tissues and placed in a 5 ml organ bath at 37°C containing oxygenated physiological salt solution of the following composition (mM): NaCl 137, KCl 2.68, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9 and glucose 5.5. The atrium was connected, under a resting tension of 5 mN, to an isometric force transducer connected to a Basile 7050 Unirecord. The atrium was stimulated by means of two wire platinum electrodes placed at the top and the bottom of the organ bath connected to a GRASS S11 stimulator. The atria were driven at a frequency of 3 Hz (0.5 ms pulse width, maximal voltage). After a 2 h equilibration period, a train of stimuli was delivered, at 15–20 min intervals, at a frequency of 10 Hz for 2.5 s (1 ms pulse width, 60 V) by means of a second stimulator, which superimposed on the stimuli delivered by the first stimulator. During the train, the atria exhibited a flutter-like response, followed, at the end of the train, by a short (4–5 s) period of increased contractility (poststimulus potentiation or postextrasystolic potentiation) while the neurogenic inotropic response to the train of stimuli appeared 15–30 s and peaked 60–120 s after the end of the train of stimuli. This stimulation produces, in left atria from reserpinized guinea-pigs and in the presence of atropine, a delayed positive inotropic response mediated by antidromic invasion of capsaicin-sensitive sensory nerve terminals.

Guinea-pig bronchi

Zig-zag strips of the main guinea-pig bronchus were prepared as described previously (Maggi *et al.*, 1988b; Giuliani *et al.*, 1989), placed in 5 ml organ baths containing oxygenated (96% O_2 and 4% CO_2) Krebs solution at 37°C plus atropine (1 μM) and indomethacin (5 μM). Tension was recorded by means of an isotonic transducer (load 5 mN) connected to a Basile 7050 pen recorder. Electrical field stimulation was obtained as described above for the guinea-pig atria, using trains of stimuli (2–5 Hz, 60 V, 0.5 ms pulse width for 10 s).

The responses to electrical field stimulation, capsaicin or neurokinin A were expressed as % of the maximal response to KCl (40 mM, added to the bath).

Statistical analysis

Each value is mean ± standard error (s.e.) of the mean. Statistical analysis was performed by means of Student's *t* test for paired or unpaired data, when applicable. Regression analysis was performed by the least squares method. EC_{50} and 95% confidence limits (c.l.) were calculated accordingly.

Drugs

Drugs used were: capsaicin and indomethacin (Sigma), atropine HCl and reserpine (Serva), neuropeptide Y, rat calcitonin gene-related peptide and neurokinin A (Peninsula).

Results

Guinea-pig left atria

NPY produced *per se* a small transient positive inotropic effect (threshold concentration 100 nM) followed by a small negative inotropic effect which peaked at 5 min (Figure 1). At 1 μM, the positive and negative inotropic changes produced by NPY averaged $+9.9 \pm 2$ and $-12 \pm 3\%$ variation of the resting tension ($n = 10$).

In the electrically-driven left atria from reserpine-pretreated guinea-pigs and in the presence of atropine, application of a train of stimuli (10 Hz, 1 ms, 60 V for 2.5 s) produced a delayed positive inotropic response ($57 \pm 7\%$ increase over resting values, $n = 13$) which was fairly reproducible at 15–20 min intervals without any sign of fading. This response has been characterized previously (Goto *et al.*, 1987; Saito *et al.*, 1987; Maggi *et al.*, 1988b; 1989a; Giuliani *et al.*, 1989) as being dependent upon activation of capsaicin-sensitive nerves which release a CGRP-like transmitter responsible for the inotropic effect. NPY produced a concentration- (10 nM–1 μM, 5 min before) dependent inhibition of these non-adrenergic non-cholinergic inotropic responses (Figures 1 and 2). The effect of NPY was fully reversible 30 min later, after thorough washing out (Figure 1). The EC_{50} for this inhibitory effect was 63 nM (35–143 nM, 95% c.l.).

By contrast, NPY had no inhibitory effect on inotropic responses produced by application of capsaicin (30 nM, $n = 5$) or exogenous CGRP (3 nM, $n = 5$) (Figure 3), at doses matched to produce an inotropic response similar to that observed in response to electrical field stimulation. These concentrations of capsaicin and CGRP were submaximal. In the present experimental conditions, maximal inotropic effects were obtained with 1 μM capsaicin and 0.1 μM CGRP. The concentrations used for the NPY experiments (30 nM for capsaicin, 3 nM for CGRP) produced a response which averaged 24 ± 4 and $21 \pm 5\%$ of the maximum for capsaicin and CGRP, respectively ($n = 5$ for each agent).

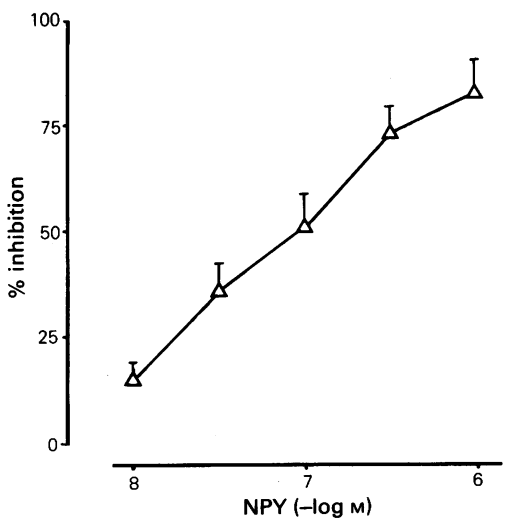

Figure 2 Concentration-dependent inhibition by neuropeptide Y (NPY) of the delayed positive inotropic response produced by electrical train field stimulation in the guinea-pig isolated left atria. Each value is mean of 6 experiments with s.e. shown by vertical bars.

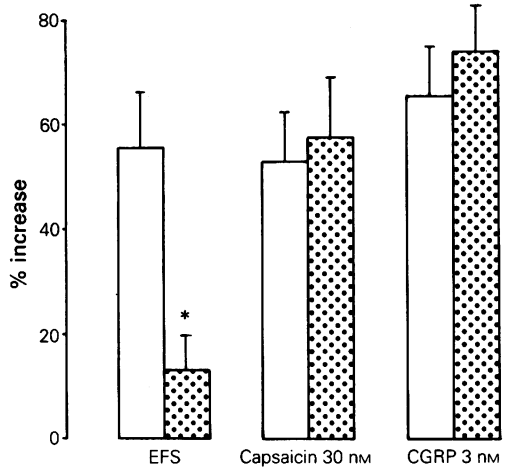

Figure 3 Comparison of the positive inotropic response produced in the guinea-pig isolated left atria by electrical field stimulation (EFS, 10 Hz, 60 V, 1 ms for 2.5 s), capsaicin or exogenously administered calcitonin gene-related peptide (CGRP). Open columns = controls; stippled columns = 5 min after addition of neuropeptide Y (1 μM). * Significantly different from controls $P < 0.01$.

Guinea-pig bronchi

In the presence of atropine (1 μM) and indomethacin (5 μM) electrical field stimulation produced frequency-related, slowly developing non-adrenergic non-cholinergic (NANC) contractions due to antidromic activation of capsaicin-sensitive nerves (Szolcsányi & Barthó, 1982; Barthó *et al.*, 1987; Maggi *et al.*, 1988b; Giuliani *et al.*, 1989). NPY (0.1 μM) did not affect tension. The NANC contractile response to electrical field stimulation (2–5 Hz, 60 V, 0.5 ms for 10 s) was significantly reduced by NPY (0.1 μM, 5 min before) by about the same amount (50–60% inhibition) at both frequencies (Figure 4). NPY (0.1 μM, 5 min) did not affect the response to exogenous neurokinin A (2 nM) or capsa-

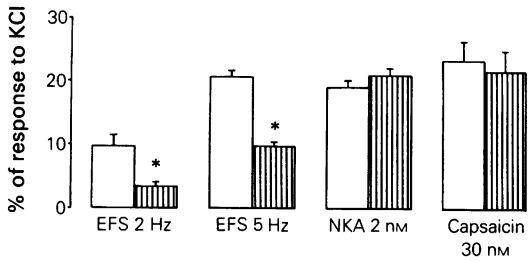

Figure 4 Comparison of the positive inotropic response produced by electrical field stimulation (EFS, 2–5 Hz, 0.5 ms, 60 V for 10 s), neurokinin A (NKA) or capsaicin in the guinea-pig isolated bronchus (atropine and indomethacin in the bath). Open columns = control; lined columns = 5 min after addition of neuropeptide Y (0.1 μM).

icin (30 nM) (Figure 4). These concentrations of neurokinin A and capsaicin were submaximal. In the present experimental conditions, maximal responses were obtained at 3 μM neurokinin A and 1 μM capsaicin which averaged 80 ± 4 and $82 \pm 5\%$ of the maximal response to KCl (40 mM, $n = 4$ for each agent). The concentrations of neurokinin A (2 nM) and capsaicin (30 nM) used for the NPY experiments were matched to produce a contractile response similar to that observed in response to electrical field stimulation at 5 Hz (about 20% of the maximal response to KCl, Figure 4).

Discussion

Using appropriate experimental conditions it is possible to elicit, in the guinea-pig isolated left-atria and bronchi, motor responses produced by antidromic activation of capsaicin-sensitive afferents (Szolcsányi & Barthó, 1982; Goto *et al.*, 1987; Saito *et al.*, 1987; Barthó *et al.*, 1987; Maggi *et al.*, 1988b; 1989a). These responses are highly reproducible and appear particularly suitable for studying the modulatory influence of substances on the excitability of the sensory nerves.

Present findings indicate that NPY exerts a potent inhibitory effect on motor responses produced when the capsaicin-sensitive nerves were activated by electrical field stimulation but not when they were activated by capsaicin. As NPY did not affect responses to CGRP (left atria) or neurokinin A (bronchi), the putative endogenous mediators of the responses produced by activation of capsaicin-sensitive nerves, present data strongly suggest a prejunctional action of NPY. This conclusion is consistent with the well known neuromodulatory action of NPY on autonomic nerves (Lundberg *et al.*, 1984; Wahlestedt *et al.*, 1986; Potter, 1988).

We have previously shown (Maggi *et al.*, 1988b,c; 1989a) that the activation of peripheral endings of capsaicin-sensitive sensory nerves by electrical field stimulation and capsaicin differ in their sensitivity to ω-conotoxin fraction GVIA. In particular, ω-conotoxin suppressed the responses produced by electrical field stimulation while leaving the responses to capsaicin unaffected. From these findings, we proposed that the responses produced by these two stimuli would be dependent on (electrical field stimulation) and independent of (capsaicin) activation of ω-conotoxin-sensitive voltage-dependent calcium channels. A selective inhibition of sensory nerve excitability when they are activated by field stimulation but not when they are activated by capsaicin has been reported previously for opioids (Barthó *et al.* 1987) and for the neuropeptide galanin (Giuliani *et al.*, 1989). This may reflect a peculiar mode of action of capsaicin which is actually capable of concentrating extracellular calcium within sensory neurones (Wood *et al.*, 1988) in such a way that even μmolar calcium concentrations in the medium are sufficient to sustain a full release of sensory neuropeptides from peripheral endings of capsaicin-sensitive nerves (Maggi *et al.*, 1988d; 1989b).

NPY has been shown to inhibit calcium currents in rat dorsal root ganglia and abolish the depolarization-coupled (high K medium) release of substance P (Ewald *et al.*, 1988; Walker *et al.*, 1988). As NPY selectively inhibits the responses produced via the ω-conotoxin-sensitive mode of activation of sensory fibres (Maggi *et al.*, 1988b), the present findings agree with those of Walker *et al.* (1988) suggesting inhibition of voltage-sensitive calcium channels as a likely mechanism for the prejunctional inhibitory action of NPY on excitability of primary sensory neurones.

NPY is released from sympathetic nerves in the peripheral nervous system, particularly at high frequencies of stimulation (Lundberg *et al.*, 1986; 1989). Thus we cannot exclude the possibility that endogenous NPY might have been released during train field stimulation e.g. that the effects of exogeneous NPY observed in this study were superimposed on a background of endogenous peptide. This might well apply to the experiments in the bronchi, while it seems less likely for experiments in the atria because these preparations were obtained from reserpine-pretreated animals. In fact, reserpine pretreatment was shown to produce a marked reduction of the NPY level in the guinea-pig atria (Lundberg *et al.*, 1985; Franco-Cereceda *et al.*, 1987).

The present findings suggest a possible mode of interaction between sympathetic and sensory nerves at the peripheral level (cf. Wall & Gutnick, 1974; Levine *et al.*, 1986), that is, prejunctional inhibition of sensory nerve excitability by NPY. Further studies are needed to assess whether sensory impulse

generation and/or transmission are affected by this neuropeptide concomitantly with inhibition of the 'efferent' function of these sensory nerves.

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