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Inhibition of sympathetic cholinergic vasodilatation by a selective NPY Y_2 receptor agonist in the gracilis muscle of anaesthetised dogs

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Abstract

Neuropeptide Y (NPY) is known to be co-stored and co-released from sympathetic nerve terminals. In the cardiovascular system NPY acts on two main receptor subtypes. At the postjunctional or Y_1 receptor NPY causes constriction directly in addition to potentiating other vasoconstrictor agents. NPY acting at the prejuctional, or Y_2 receptor, inhibits the release of neurotransmitter from autonomic nerve terminals. In these experiments we used the selective Y_2 receptor agonist *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 to examine the role of NPY in the modulation of sympathetic vascular control in skeletal muscle in anaesthetised dogs. No systemic pressor or local constrictor activity was observed in response to *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 administration, therefore allowing us to examine the neuroinhibitory actions of NPY in the absence of direct vascular effects on blood flow. Stimulation of the sympathetic nerves to the gracilis muscle engages both sympathetic cholinergic and sympathetic adrenergic fibres and produces an initial vasodilatation followed by a slower vasoconstriction. Nerve evoked vasodilatation was inhibited by over 50% in the presence of the selective NPY Y_2 agonist *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36. This dilatation was abolished by atropine, confirming its cholinergic nature. *N*-Acetyl[Leu^{28}, Leu^{31}]NPY24–36 was found to have no effect on nerve evoked vasoconstriction. The results demonstrate a NPY Y_2-receptor mediated inhibition of nerve evoked sympathetic cholinergic vasodilatation but not of sympathetic vasoconstriction. © 1998 Elsevier Science B.V.

Keywords: NPY; NPY Y_2 agonist; Sympathetic vasoconstriction; Cholinergic vasodilatation

1. Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide that is widely distributed throughout the central and peripheral nervous system. It is colocalised with noradrenaline in sympathetic nerves to the cardiovascular system. In 1986 Hakanson and Wahlestedt suggested the presence of two NPY receptors at the sympathetic neuroeffector junction. NPY Y_1 receptors are suggested to be located postsynaptically and mediate constriction and potentiation of constriction in isolated vascular preparations, an effect that requires the full NPY molecule [29]. In contrast, an NPY Y_2 receptor was described in the rat vas deferens, located presynaptically on sympathetic nerve terminals which when activated by either NPY and or C-terminal fragments of NPY, typically NPY 13–36, results in the inhibition of neurotransmitter release. NPY 13–36 was described as having no activity at Y_1 receptors in this study [29]. In addition to the suppression of neurotransmitter release from sympathetic nerve terminals, NPY has been shown to inhibit the effectiveness cardiac vagal action on the heart [12,15], an effect that is mimicked by high frequency stimulation of cardiac sympathetic nerves in the presence adrenoceptor blockade [22–24] and this was shown to be a presynaptic one [24].

In 1989 an anaesthetised rat model was described which allowed postsynaptic pressor activity (a Y_1 action) and presynaptic inhibition of cardiac vagal activity (a Y_2 action) to be measured simultaneously [26]. In this model intravenous injection of the NPY Y_2 agonist, NPY13–36, showed significant ability to inhibit vagal activity (Y_2 activity) and significant pressor or Y_1 activity [17]. The demonstration of a persistent Y_1 action questions the selectivity of the NPY 13–36 fragment, a finding that is supported in other system where the NPY 13–36 has been shown to evoke vasoconstriction in a number of vascular beds [13,18–20]. In contrast, the NPY analog, *N*-

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acetyl[Leu^{28}, Leu^{31}]NPY24–36 is as potent as NPY at inhibiting vagal activity and shows no significant pressor activity in the rat model [25]. The NPY Y_2 selectivity has been confirmed both in cultured cells expressing Y_1 and Y_2 receptors [25] and the nasal mucosa of the dog where *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 inhibited cholinergic vasodilatation without evoking local vasoconstriction or systemic pressor activity [14].

In this paper we have looked at the gracilis muscle of the dog in order to determine the potential role of NPY in the local regulation of sympathetic activity in skeletal muscle. The sympathetic innervation of skeletal muscle displays two distinct groups, one an adrenergic vasoconstrictor fibre containing NPY [16] and the other a cholinergic vasodilator fibre [1,2,28]. To date there has been no examination of the role of NPY in the regulation of sympathetic cholinergic vasodilator fibres. Therefore it was of interest to see if this population of cholinergic vasodilator fibres are inhibited by the presence of the NPY Y_2 receptor agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36, given that the same agonist effectively inhibits cholinergic vasodilator fibres in the nasal mucosa [14].

The effect of NPY on the release of transmitter from constrictor fibres in the gracilis muscle has been previously explored in two ways. Two apparently separate studies by Pernow et al. have reported essentially the same depression of nerve evoked overflow of tritiated noradrenaline in the presence of constrictor doses of NPY [16,21]. This depression of noradrenaline overflow could not be translated into a depression of the constrictor activity when sub-constrictor doses of NPY were used in the same preparation [27]. Revington and McCloskey (1987) demonstrated that NPY potentiated the constrictor effects of exogenous noradrenaline and gracilis nerve stimulation to the same degree, suggesting predominant Y_1 or postsynaptic activity. Due to the similarity of potentiation of the nerve evoked and noradrenaline evoked constrictions the authors concluded that it was doubtful that there was any presynaptic inhibitory effect of NPY [27]. Similar difficulties in separating the pre- and postsynaptic actions of NPY have been demonstrated in the pithed rat model following preganglionic stimulation of the spinal sympathetic ganglia. Initial experiments demonstrated that the pressor response evoked by nerve stimulation [3] was enhanced in the absence of reduced transmitter release from sympathetic nerves [31]. Subsequent experiment in the same model demonstrated that, while NPY enhanced both pressor and cardiac acceleration evoked by preganglionic nerve stimulation, there was an apparent reduction in the nerve evoked release of catecholamines into the circulation [4].

We also examined the effects of the NPY Y_2 agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36, on sympathetic vasoconstriction in an attempt to show an inhibition of sympathetic vasoconstriction, without the complication of the postsynaptic effect of NPY mediated constriction or potentiation of constriction allowed by the use of our selective NPY Y_2 receptor agonist.

2. Methods

Experiments were carried out on 15 dogs of both sexes weighing between 6 and 20 kg. Institutional Animal Care and Ethics Committee approval was obtained. The dogs were anaesthetised with a bolus intravenous injection of sodium pentobarbitone sodium (35 mg kg^{-1} Nembutal, Boehringer Ingelheim, Australia) and maintained on an infusion of 2–3 mg kg^{-1} h^{-1}. The dogs were ventilated through a tracheal cannula using a Harvard positive pressure ventilator. The left jugular and right femoral veins were cannulated for the administration of drugs and anaesthetic, respectively. The right femoral artery was cannulated and attached to a Statham P23 pressure transducer for the measurement of arterial blood pressure. The left gracilis muscle was exposed to reveal the main arterial supply and a transonic blood flow probe was placed around the artery. The blood flow signal was monitored by a transonic blood flow meter (T206, Transonic System, NY) and recorded on a Grass polygraph (model 79D, Grass Instruments, Quincy) together with arterial blood pressure and pulse interval (the period between successive beats of the heart, derived from the electrocardiogram).

2.1. Nerve evoked activity

The gracilis nerve was isolated, cut and placed over bipolar platinum electrodes connected to an isolated square wave stimulator (model S88, Grass Instruments, Quincy). The nerve motor threshold was determined (usually 1.5–2 V, 1 ms). Prior to paralysis a deep plane of surgical anaesthesia was established and confirmed. The dog was paralysed with a bolus dose of pancuronium bromide (Pavulon; Astra, Sweden, 80 μg kg^{-1}) followed by an intravenous infusion (80 μg kg^{-1} h^{-1}) to prevent muscle contraction when the nerve was stimulated. Throughout the period of paralysis anaesthetic infusion was continued and the level of anaesthesia was monitored through continuous recordings of blood pressure and heart rate.

The experiment did not begin until after a 30–45 min period of stabilisation following surgery and establishment of neuromuscular paralysis. The nerve to the gracilis muscle was then stimulated at 20 times the motor threshold and the response observed. The gracilis nerve was stimulated at 1 Hz with a duration sufficient to evoke a marked and reproducible change in blood flow for up to 30 s. The dose of the NPY Y_2 receptor agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 (70 μg kg^{-1}:40 nmol kg^{-1}; Chiron Mimotopes, Melbourne) was used. It was twice the dose found to inhibit parasympathetic vasodilatations in the nasal mucosa and inhibit parasympathetic activity in the heart [14,25].

2.1.1. Sympathetic (cholinergic) vasodilatation

In an initial four dogs the nerve evoked vasodilatation was studied in the presence of varying degrees of vasoconstrictor activity. In two dogs the effect of the NPY Y_2 receptor agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36, was tested on the initial vasodilatation which was followed by a more pronounced vasoconstriction. In the following two dogs nerve stimulation initially elicited vasoconstriction only, however an increase in flow could be demonstrated following intravenous injection of the α-adrenoceptor antagonist phentolamine (bolus 500 μg kg^{-1} followed by an infusion of 50 μg kg^{-1} h^{-1}; Regitine: Ciba-geigy, Switzerland). The effect of the NPY Y_2 agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36, was also tested on the mixed vasodilator/vasoconstrictor response in these two dogs.

Given the mixed nature of the response and pretreatments used in these dogs, the effects of the NPY Y_2 agonist were tested in a further five animals, in which a dose of guanethidine sufficient to block the vasoconstriction evoked by nerve stimulation was administered (3–12 mg kg^{-1}) [6,9]. In four of the five animals reproducible increases in flow were evoked by a single shock of 1 ms duration. In the fifth animal two shocks, at 1 Hz, were required to achieve a reproducible increase in blood flow. The neurally evoked changes in flow were tested before and after the administration of 70 μg kg^{-1} (40 nmol kg^{-1}) *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 (Chiron Mimotopes, Melbourne).

2.1.2. Sympathetic vasoconstriction

In five dogs vasoconstrictor activity evoked by nerve stimulation was studied. In three of these, where nerve stimulation evoked no clear element of vasodilatation, nerve evoked vasoconstriction was tested before and after the administration of 40 nmol kg^{-1} of *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36. In a further two dogs, which had previously been used to study vasodilator responses, a bolus dose of atropine (0.1–0.2 mg kg^{-1}: atropine sulfate; Astra, Sweden) was administered and the effect of *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 on evoked vasoconstriction determined: neither of these dogs received any other pre-treatment. When an enhanced vasoconstrictor effect was observed in response to the NPY analog, an additional dose of atropine (1 mg kg^{-1}) was administered before re-testing *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 on the constrictor response.

2.2. Agonist induced (cholinomimetic) vasodilatation

In a further three animals the effect of the Y_2 agonist on vasodilatation induced by an exogenous cholinomimetic was examined. In these animals the gracilis nerve was cut but was not stimulated. A small arterial cannula was introduced into a side branch of the gracilis artery for the introduction of the cholinomimtic agent methacholine (acetyl-β-methylcholine bromide; Sigma, Australia). A dose of methacholine was chosen that produced a submaximal increase in gracilis blood flow, similar in magnitude to the vasodilatations observed following nerve stimulation, in the absence of systemic activity (usually 1–100 ng).

2.2.1. Data analysis

In order to compare data from different animals, gracilis artery blood flow and mean arterial blood pressure data were converted to vascular conductance. The change in conductance upon nerve stimulation was then calculated as a percent change in conductance to the conductance immediately prior to stimulation. All trial stimulations repeated after the administration of *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 were expressed as a percentage of the control response (see below). The extent of inhibition after *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 administration, was determined in each animal and reported as mean $\pm$ standard error of the mean. The data were then compared by a one way analysis of variance followed by Dunnett's multiple comparison test [30]. Where nerve evoked vasodilatation had been observed, a bolus dose of atropine was administered (1 mg kg^{-1}) in all cases this abolished the vasodilatation, confirming its cholinergic nature.

2.2.2. Equations

In order to compare data from different dogs, gracilis blood flow and mean arterial blood pressure (MBP) data were converted to vascular conductance (C). The effect of gracilis nerve stimulation (s) was expressed as the % change in conductance (%C) above or below conditions prior to stimulation (unstimulated, u). Evoked changes in conductance obtained after the *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 administration (test, t) were normalised to the respective control response in each dog (c). The maximal inhibition was calculated as 100 − (% of control) in each dog.

$$\text{conductance:}\quad C = \frac{\text{flow (ml/min)}}{\text{MBP (mmHg)}},$$

$$\text{\% change in conductance:}\quad \%C = \frac{C_u - C_s}{C_u} \times 100,$$

$$\text{\% of control response:}\quad = \frac{C_c - C_t}{C_c} \times 100,$$

$$\text{\% inhibition:}\quad = 100 - (\text{\% of control response}).$$

3. Results

3.1. Nerve evoked activity

3.1.1. Sympathetic (cholinergic) vasodilatation

When the nerve evoked response was examined following pretreatment with guanethidine only vasodilatation was

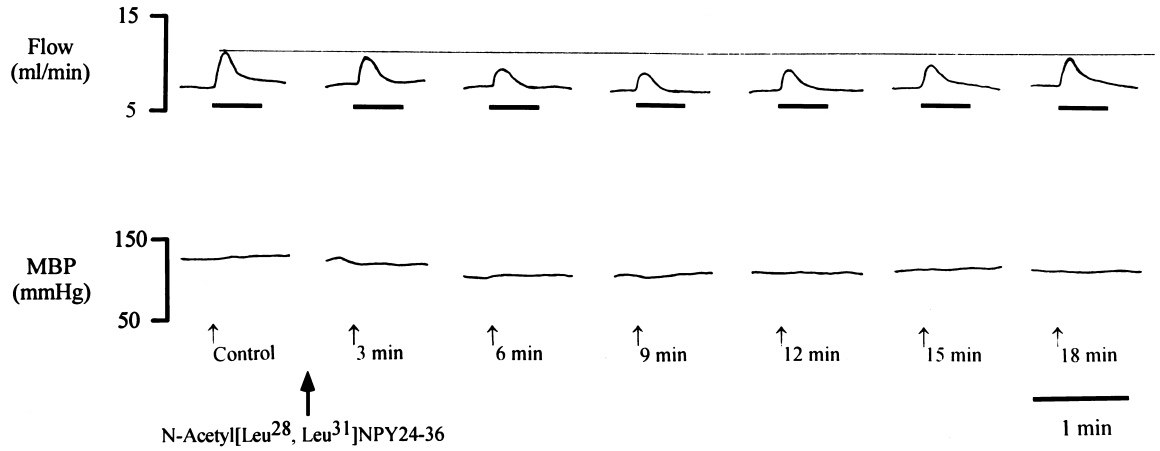

Fig. 1. Record showing the effect of the NPY Y_2 agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 (40 nmol kg^{-1}), on vasodilatations evoked by electrical stimulations (————) of the sympathetic nerve (upper trace). Mean arterial blood pressure is shown on the lower trace. Maximal inhibition was observed 6 min after the administration of the NPY Y_2 agonist with the response recovering to control levels at 18 min.

observed. While the response to a nerve stimulation was reproducible in any given animal, the response varied between animals with conductance increasing by 30 to 120%. The average increase in conductance was $73 \pm 17\%$. In each animal the injection of the NPY Y_2 agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36, produced an inhibition of vasodilator activity. When each animal was compared to its control response a decrease in vasodilator activity was observed in the 6–15 min following injection of the NPY Y_2 agonist. Fig. 1 shows a polygraph trace from one animal. In the grouped data from five dogs nerve evoked vasodilatation was reduced by $26 \pm 5\%$, $24 \pm 9\%$ and $25 \pm 8\%$ in the sixth, ninth and twelfth minutes following injection of the NPY Y_2 agonist respectively (Fig. 2; $P < 0.05$). Given that the time to maximal inhibition varied from animal to animal, maximal inhibition occurred 10 ± 2 min after the injection of the NPY Y_2 agonist and amounted to a $37 \pm 4\%$ inhibition of vasodilator activity. In all animals the vasodilator response to nerve stimulation was abolished by the injection of atropine, confirming its cholinergic nature.

Vasodilator responses to nerve stimulation were also observed in four other dogs. In two dogs the responses were seen without pretreatment and were followed by vasoconstriction. In a further two animals the initial vasodilatation was only evident following pretreatment with phentolamine. In all these dogs the increase in flow was abolished in the presence of atropine and was inhibited by the NPY Y_2 receptor agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 (data not shown).

Because of the presence of both sympathetic constrictor (noradrenergic) and dilator (cholinergic) fibres in the nerve supply to the skeletal muscle, blood flow responses to nerve stimulation commonly show evidence of the actions of both types of innervation. Typically, there is a brisk vasodilatation (abolished by atropine, see above) followed by a slower vasoconstriction. At the conclusion of stimulation, there typically occurred a period of increased flow that varied from stimulation to stimulation (e.g. Fig. 3). The administration of the NPY Y_2 agonist *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 produced no local vasoconstrictor activity or systemic pressor activity. However, in four of the nine dogs tested, a fall in mean arterial blood pressure was sometimes observed in response to a single dose of *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 (e.g. Fig. 1). This fall in

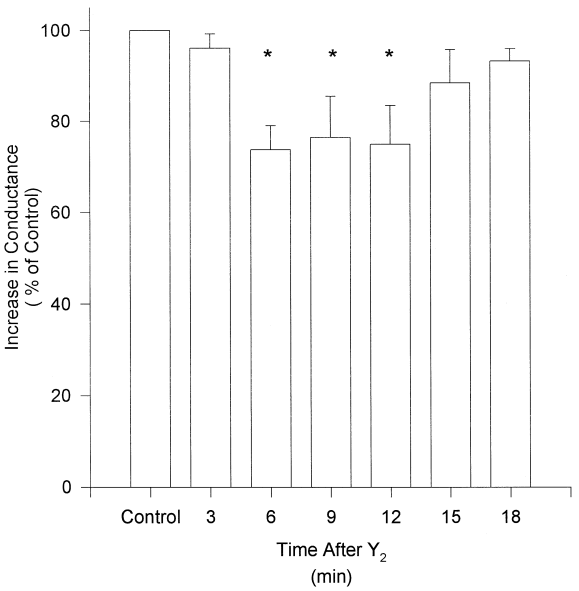

Fig. 2. Mean increase in vascular conductance resulting from the stimulation of the sympathetic nerve to the gracilis muscle in six dogs. At 6, 9 and 12 min following injection of the NPY Y_2 agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 (40 nmol kg^{-1}), vasodilator activity was reduced by ~25% when compared to the control response. All data are presented as the mean ± standard error of the mean ($^{*}P < 0.05$, $n = 5$).

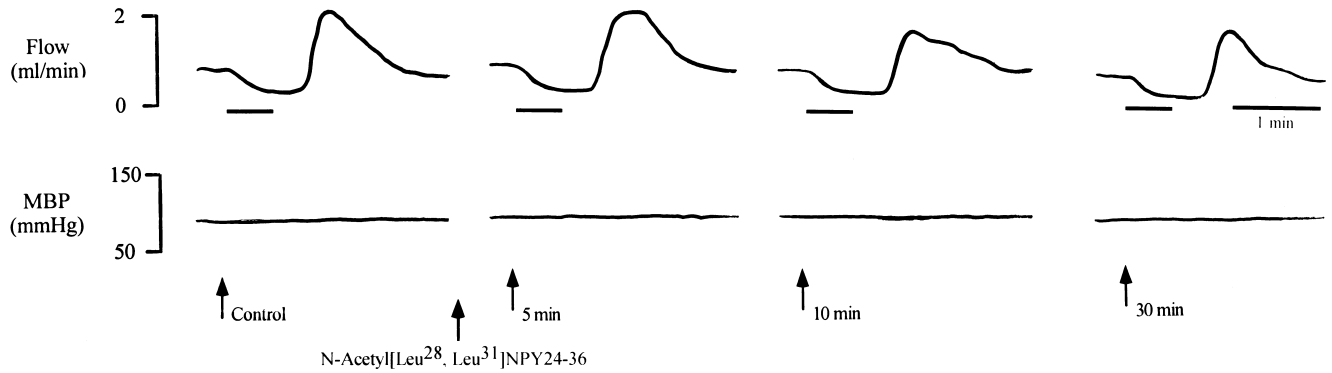

Fig. 3. Record showing the lack of effect of the NPY Y_2 agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 (40 nmol kg^{-1}), on vasoconstriction evoked by electrical stimulation (————) of the sympathetic nerve (upper trace). Mean arterial blood pressure is shown on the lower trace. Sympathetic stimulation decreased blood flow by approximately 63% and this decrease in flow was not affected by the NPY Y_2 receptor agonist. The increase in flow that followed vasoconstriction could not be related to the degree of vasoconstriction or the administration of the NPY Y_2 receptor agonist.

blood pressure was small and inconsistent and could not be related to the order of the Y_2 agonist injection.

3.1.2. Sympathetic vasoconstrictions

The effect of the NPY Y_2 receptor agonist on nerve evoked vasoconstrictions was tested in five dogs. Three of these had shown no evidence of a vasodilator response, and the other two were animals in which a mixed vasodilator/vasoconstrictor response to nerve stimulation occurred. In the three animals showing a pure vasoconstrictor response to nerve stimulation, administration of *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 had no effect on the vasoconstrictor responses. The vasoconstrictor response were also unaffected by administration of atropine (1 mg kg^{-1}), indicating that there was no cholinergic component contributing to the overall response. An example from one dog is shown in Fig. 3.

In the remaining two animals, a vasodilator component of the response to nerve stimulation was evident as an initial flow increase, followed by a variable flow reduction as the constrictor response became apparent. Administration of *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 reduced the initial flow increase. After atropine (0.1–0.2 mg kg^{-1}) the initial vasodilatation was greatly attenuated and the constrictor activity became reproducible. At this stage *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 administration was associated with enhanced constrictor activity. We presumed that a residual vasodilator response, reduced by the NPY Y_2 agonist, had allowed the constrictor effect to become more apparent. This was confirmed by the administration of a large dose of atropine (1 mg kg^{1-}). After such doses of atropine there was no initial dilator component, and the constrictor component was then unaffected by administration of the NPY Y_2 receptor agonist.

Increases in blood flow that occurred at the end of sympathetic stimulation varied from stimulation to stimulation. The variability of the dilatation following constriction made it impossible to determine an effect of the Y_2 agonist on this response.

3.2. Agonist (cholinomimetic) induced vasodilatation

In three dogs local intraarterial injection of methacholine (1–100 ng) increased conductance by 174 ± 70%.

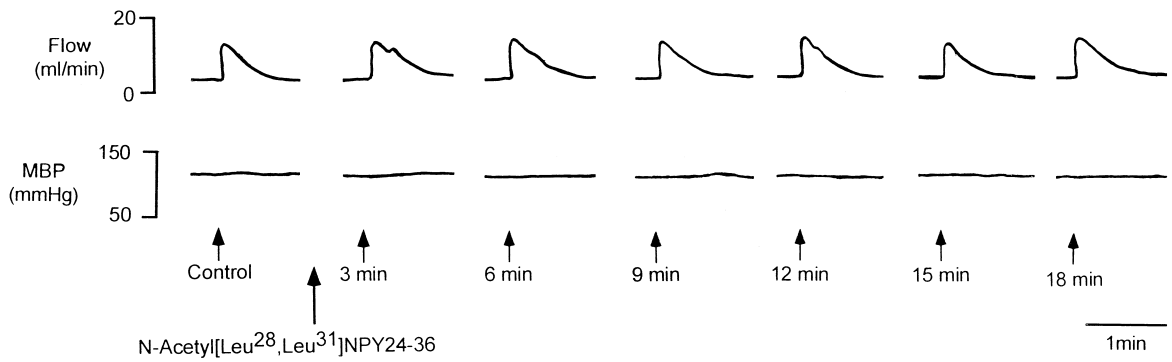

Fig. 4. Record from an anaesthetised dog showing no effect of the NPY Y_2 agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 (40 nmol kg^{-1}), on vasodilatation evoked by local intraarterial injection of methacholine (upper trace). This dose of methacholine (50 ng) showed no systemic activity as shown in the lower trace of mean arterial blood pressure.

No inhibition of agonist induced vasodilatation was observed following administration of the Y_2 agonist *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36. An example from one dog is shown in Fig. 4.

4. Discussion

The results presented here show that the specific NPY Y_2 receptor agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36, produces an inhibition of neurally evoked vasodilatation in the vascular bed of the gracilis muscle in the anaesthetised dog. This vasodilatation was completely abolished by atropine and is therefore presumed to have been wholly cholinergic. When vasoconstriction was examined, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 was followed by increased constrictor activity in two dogs which had no pretreatment. We believe this apparent increase in constrictor activity is the result of inhibition by *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 of an underlying cholinergic vasodilatation because this effect was abolished in the presence of atropine. This finding, therefore, provides a further indication of the inhibition of sympathetic cholinergic activity by *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36. The inability of the NPY Y_2 agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36, to inhibit the methacholine induced vasodilatation indicates that the action of the NPY Y_2 agonist is a presynaptic one, inhibiting the release of neurotransmitter, rather than a postsynaptic action modifying the effectiveness of released transmitter on vascular smooth muscle.

Inhibition of cholinergic vasodilatation, by both NPY and the selective NPY Y_2 agonist *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36, has been demonstrated in the vascular bed of the nasal mucosa of the dog [14]. Furthermore, both NPY and *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 have been shown to inhibit cardiac vagal (cholinergic) activity in the rat [22,25]. The results presented here are the first demonstration of a peptide dependent inhibition of cholinergic vasodilator activity post ganglionic sympathetic fibres.

Earlier work on the gracilis muscle by Revington et al. [27], Kahan et al. [10] and Pernow et al. [21] focused on effects of NPY on nerve evoked constrictions and responses to exogenous constrictor agents. At non-pressor doses of NPY, Revington and McCloskey demonstrated a potentiation of neurally evoked pressor activity and the pressor action of exogenous phenylepherine, consistent with postsynaptic (Y_1 effect) potentiation of constrictor activity proposed by other workers [5,7]. In contrast, at constrictor doses of NPY, Pernow et al. [21] and Kahan et al. [10] reported a reduction in measured noradrenaline spillover.

In the results presented here and work in the nasal mucosa [14] the administration of *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36 was found to have no local constrictor or systemic pressor activity. In addition, the two cases of potentiation of constrictor activity presented here were abolished by atropine and can be presumed to reflect an inhibition of competing cholinergic activity rather than potentiation of postsynaptic constrictor activity. These observations clearly support the NPY Y_2 receptor selective or neural inhibitory role of *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36. However it is of interest that while cholinergic activity was inhibited sympathetic constrictor activity was unaffected despite the use of the same dose on both sympathetic subtypes, and a dosage twice that which inhibits parasympathetic activity in the nasal mucosa and heart [14,25]. The lack of suppressed sympathetic vasoconstrictor activity, in the presence of the NPY Y_2 agonist, is in contrast to the indication given by Pernow et al. [21] and Kahan et al. [10] that NPY inhibited noradrenaline release. Pernow et al. [21] noted, however, that the underlying vasoconstriction from exogenous NPY may have limited the diffusion of noradrenaline from the muscle contributing to the observed apparent inhibition of noradrenaline release. The inability of Revington and McCloskey [27] to demonstrate inhibition of sympathetic activity by NPY, together with the results presented here, make it unlikely that there is a role for NPY in the modulation of sympathetic constrictor activity in the gracilis muscle.

In the work presented here, a variable increase in flow occurred after the cessation of sympathetic stimulation. This increase in flow was observed after a sustained vasoconstriction but was not observed after a similar period of stimulation in the absence of constrictor activity. It would therefore appear that the increased flow was brought about in response to the sustained vasoconstriction as comparable stimulation of the gracilis nerve, in the absence of constrictor activity, failed to evoke increases in blood flow after the cessation of the stimulus. This observation is consistent with the sensitivity of gracilis blood flow to changes in local oxygen and metabolite concentration [8,11] and therefore can most likely be attributed to a reactive hyperemia brought about by a period of reduced blood flow.

In conclusion, the results presented here show that the selective NPY Y_2 agonist, *N*-acetyl[Leu^{28}, Leu^{31}]NPY24–36, produces an inhibition of nerve evoked activity in sympathetic cholinergic terminals but not in sympathetic adrenergic terminals. In the event of concurrent activation of sympathetic constrictor and sympathetic dilator fibres, it could be predicted that the release of NPY from the sympathetic adrenergic subset would assure dominance for the constrictor activity, by inhibiting cholinergic dilators: for sympathetic cholinergic vasodilatation to be optimal, selective activation of the cholinergic subset of fibres would be necessary.

Acknowledgements

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