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Effects of pituitary adenylate cyclase-activating polypeptide on cardiovascular and respiratory responses in anaesthetised dogs

Myfanwy J. Runcie, Lesley G. Ulman, Erica K. Potter *

Prince of Wales Medical Research Institute, Prince of Wales Hospital, Randwick 2031, Sydney, NSW, Australia

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Abstract

This study examines some of the cardiovascular and respiratory effects of pituitary adenylate cyclase-activating polypeptide (PACAP) in anaesthetised dogs. Intravenous injection of PACAP 27 caused an increase in arterial blood pressure and an increase in heart rate. The blood pressure response was significantly reduced by adrenoceptor blockade suggesting a mechanism of action mediated in part via catecholamines. The heart rate increase was unaltered by adrenoceptor blockade suggesting a direct effect of PACAP 27. PACAP 27 also caused potentiation of cardiac slowing caused by stimulation of the vagus nerve. In addition, PACAP 27 powerfully stimulated breathing. This was probably evoked by stimulation of arterial chemoreceptors, because bilateral section of the carotid sinus nerves abolished this effect. PACAP 27 had no effect on the ability of the cardiac sympathetic nerve to increase heart rate, nor on the interaction between the sympathetic and parasympathetic systems in the heart.

Keywords: Pituitary adenylate cyclase-activating polypeptide (PACAP); Blood pressure; Heart rate; Vagus; Ventilation

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) was first isolated from ovine hypothalamus. Its name derives from the observation that it is a powerful stimulant of adenylate cyclase in anterior pituitary cells in culture [1]. A 38-amino acid and a 27-amino acid form of PACAP have been described, PACAP 27 corresponding to the N-terminal 27 amino acids of PACAP 38 [2]. It is widely distributed in mammalian tissues and has been reported to affect numerous organ systems. It has been found in several areas of the brain including the hypothalamus, posterior pituitary and hippocampus [3], and in the testes, adrenal gland and the gastrointestinal tract [4]. It is present in nerve fibres around small blood vessels in the brain and lung [1,2,5] and may have a vasoregulatory role. However it has been reported to have both vasodilator and vasoconstrictor actions and these appear to be at least partially dependent on the species of animal used. PACAP has been shown to relax isolated rabbit aortic smooth muscle [6].

In anaesthetised cats, PACAP 27 produced decreases in arterial pressure at low doses and an initial decrease followed by an increase at higher doses [7]. In anaesthetised rats, both PACAP 27 and 38 caused a dose-dependent decrease in systemic blood pressure and caused concentration-dependent relaxation

* Corresponding author. Tel.: +61 (2) 3822681; Fax: +61 (2) 3822122.

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of rat tail artery precontracted with phenylephrine [8,9]. Infusion of PACAP 38 into unanaesthetised sheep caused a small decrease in arterial pressure at high doses only [10]. In one study in anaesthetised dogs, intravenous PACAP 27 and 38 have been reported to cause a decrease in mean arterial pressure at low doses and a transient decrease followed by a marked increase in arterial pressure at higher doses [11]. However, in a second study in dogs using similar dose ranges, a transient decrease and then a more sustained increase in arterial pressure at both lower and higher doses was reported [12]. PACAP 38 has been shown to be a potent vasodilator in humans in both the forearm and the skin [13] and both PACAP 27 and 38 are potent vasodilators in rabbit skin in vivo [14]. Its effects on blood vessels therefore appear to be somewhat variable and species dependent.

The effects of PACAP on heart rate are more consistent, with both forms of PACAP causing a tachycardia in dogs [12,11], sheep [10], cats [7] and rats [9]. The mechanism of this tachycardia was not examined in these studies although it has been suggested that it occurs reflexly, secondary to a drop in blood pressure [9]. However, tachycardia occurs even in those studies in which PACAP increases blood pressure [7,12].

PACAP-immunoreactive nerve fibres have been found in the tracheobronchial wall of rats, guinea pigs, ferrets, pigs, sheep and squirrel monkeys and some fibres are also located in the lungs [5]. In humans, PACAP-containing nerve fibres have been found to be abundant in the tracheal and bronchial wall [15]. Intravenous administration of both forms of PACAP 27 to anaesthetised dogs produced a dose-dependent increase in respiratory frequency [11].

In the present study, we investigated the effects of intravenous administration of PACAP 27 on femoral arterial blood pressure (BP) and heart rate (HR) in anaesthetised dogs. We also investigated the possible mechanisms for the stimulation of ventilation found in anaesthetised dogs. The effects of PACAP 27 on sympathetic and parasympathetic action at the heart were also investigated. As the effects on BP, HR and breathing occur simultaneously, these observations were collected from each dog, following a single dose of PACAP 27.

2. Materials and methods

2.1. Surgical preparation

Experiments were performed on 12 adult mixed breed dogs of both sexes weighing between 3 and 10 kg. The dogs were anaesthetised with pentobarbitone sodium (35 mg/kg i.v.). The trachea was cannulated low in the neck. One femoral artery was cannulated and a Gould Statham blood pressure transducer (P23) was connected to give a continuous blood pressure (BP) record and a femoral vein was cannulated for administration of drugs and further doses of anaesthetic. The electrocardiogram (ECG) was recorded through needle electrodes and displayed on a storage oscilloscope. The beat-by-beat pulse interval (PI, the time between successive heart beats) was measured from the ECG after processing with Neurolog (Digitimer, UK) modules. Temperature was maintained in the range 36–38°C.

Both vagus nerves were cut high in the neck and the peripheral end of the right vagus nerve was prepared for supramaximal stimulation (~ 50 V 1 ms 1–2 Hz) using a Grass S88 stimulator (with stimulus isolation unit). In 4 animals, the right cardiac sympathetic nerve was prepared as described previously [16] and also stimulated (12–15 V, 1 ms) using a Grass S88 stimulator. Both lingual arteries were cannulated beyond the carotid bifurcation, with cannulae tips facing towards the bifurcation.

A record of tracheal air flow was obtained by passing a wide (3 mm i.d.) nylon tube into the trachea and measuring the pressure drop across this catheter by connecting it to a Grass 5PT5A volumetric pressure transducer. A signal which was proportional to tidal volume was obtained by electronic integration of the flow signal using a Grass 7P10B integrator. Blood pressure, pulse interval, tracheal air flow and tidal volume were recorded on a Grass polygraph.

2.2. Drugs used

PACAP (PACAP 27, 38; Peninsula, USA or Auspep, Australia); Phentolamine (Regitine, Ciba–Geigy, Australia Ltd.; 400 μg/kg/h); phenylephrine (Neosynephrine, Winthrop Laboratories, Australia; dose given to increase BP by 50 mmHg, usually

3–20 μg); propranolol (Inderal, ICI; 1.5 mg/kg); isoprenaline (Isuprel, Winthrop Laboratories, Australia; 2 μg). All drugs were dissolved in isotonic saline. Isotonic saline had no significant effect on any parameter tested when administered alone.

2.3. Administration of adrenoceptor antagonists

All parameters recorded were measured before and after administration of adrenoceptor antagonists. Propranolol (1.5 mg/kg) was given to block β-adrenoceptors and the effectiveness of this blockade was confirmed by the lack of effect of a dose of isoprenaline (2 μg) which had previously been selected because it caused a decrease in PI of 70–100 ms. Phentolamine (400 μg/kg/h) was given to block α-adrenoceptors and the effectiveness of this blockade was confirmed by the lack of effect of a dose of phenylephrine (3–20 μg) which had previously been selected because it caused an increase in BP of approximately 50 mmHg. After administration and test of the adrenoceptor antagonists, a non-intervention period of 10 min was allowed before further administration of PACAP 27.

2.4. Experimental protocol

For each animal, a dose of PACAP 27 that had a submaximal pressor effect of the order of 50 mmHg, was selected. This dose varied between animals, but was in the range 0.25 to 1.25 μg/kg (80–477 pmol/kg). Once a dose was selected, all subsequent injections of PACAP 27 in that animal were always at that same dose.

PACAP 27 was given intravenously and its effects on blood pressure, resting heart rate and breathing were recorded ($n = 12$). Repeated administrations of the same dose of PACAP 27 (30–45 min apart) had similar effects on BP, HR and ventilation – there was no evidence of tachyphylaxis on any parameter tested when using this interdose interval. Simultaneous α- and β-adrenoceptor blockade was carried out to see whether the observed effects were due to noradrenaline on the heart (β-blockade) or on blood vessels (α-blockade). PACAP 27 was again given and effects observed.

The effect of PACAP 27 on vagal slowing of the heart was also examined ($n = 10$). The vagus nerve was stimulated at a frequency sufficient to increase pulse interval by approximately 200 ms, a submaximal change in this variable (1–6 Hz, $n = 5$), or at 3 frequencies between 1 and 6 Hz ($n = 5$). This test was done following the initial observations on BP, HR and ventilation and was repeated after α- and β-adrenoceptor blockade. We also examined the effect of PACAP 27 on the well-documented sympathetic–parasympathetic interactions known to occur in the heart ($n = 4$): cardiac sympathetic nerve stimulation causes prolonged non-adrenergic inhibition of subsequent vagal action, an effect thought to be due to the release of NPY [17]. The effect of PACAP 27 was examined on this interaction and on the increase in heart rate to sympathetic stimulation ($n = 3$).

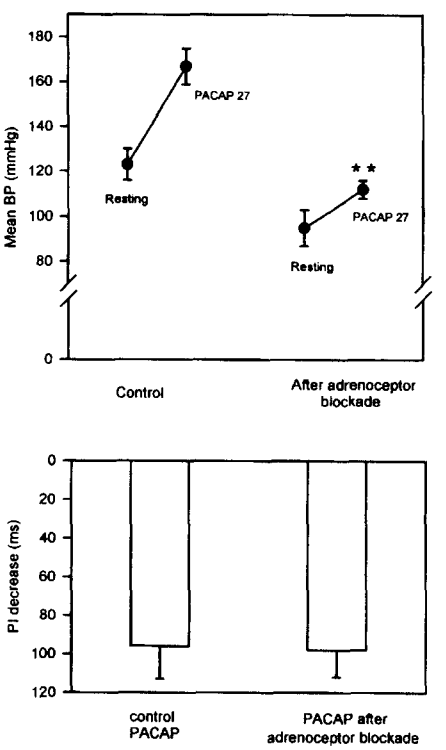

Fig. 1. Effects of PACAP injection are shown before and after adrenoceptor blockade with propranolol and phentolamine on blood pressure (BP; mmHg) in the top graph, and on pulse interval (PI; ms) in the bottom graph. Control injection of PACAP causes an increase in blood pressure which is significantly reduced by adrenoceptor blockade. Adrenoceptor blockade has no effect on the decrease in pulse interval caused by PACAP. (** $P < 0.01$; paired t-test).

Ventilation was calculated from integration of the tracheal flow signal. The effect of PACAP 27 on ventilation was measured before and after α- and β-adrenoceptor blockade and then again after bilateral ablation of carotid sinus nerves. Effectiveness of carotid sinus nerve section was tested by giving rapid retrograde injections of warmed, saline equilibrated with 100% CO_2 into the carotid bifurcation through the cannulae in the lingual arteries. When given in expiration this stimulates a breath by an action on carotid chemoreceptors [18] and this effect is abolished after carotid sinus nerve section.

2.5. Statistics

The effects of PACAP 27 on blood pressure and resting heart rate before and after adrenoceptor blockade were compared on an experiment-by-experiment basis using paired t-tests. Vagal slowing before and after PACAP 27 was compared using a paired t-test, first in the absence and then in the presence of adrenoceptor antagonists. For ventilation, the control ventilatory levels, those after PACAP 27 prior to adrenoceptor blockade, those after PACAP 27 in the presence of adrenoceptor blockade and those after carotid sinus nerve section were compared using one-way repeated measures analysis of variance with Student–Newman–Keuls tests for multiple comparisons.

3. Results

3.1. Blood pressure and pulse interval

In all animals, a standard dose of PACAP 27 (see Section 2) increased systolic, diastolic and mean blood pressures, and decreased PI (i.e., increased heart rate). Systolic and diastolic pressures rose and fell in parallel. Mean BP increased by 47 ± 8 mmHg ($n = 12$) and pulse interval decreased by 96 ± 17 ms ($n = 11$). After adrenoceptor blockade, resting mean pressure was 95 mmHg (range 33–137 mmHg, compared to a mean pressure of 120 mmHg – range 78–165 mmHg, prior to blockade). The standard dose of PACAP 27 increased BP by 18 ± 4 mmHg ($P < 0.01$) and decreased pulse interval by 98 ± 14 ms (n.s.). The results for blood pressure are shown graphically in Fig. 1. PACAP 38 increased BP and decreased PI to similar levels as PACAP 27 in the two animals in which both peptides were administered.

3.2. Vagal stimulation

PACAP 27 potentiated the effect of vagal nerve stimulation on pulse interval in all animals tested ($n = 10$). The increase in vagal effectiveness for a standard submaximal stimulus frequency was $42 \pm 5\%$ ($P < 0.001$; unpaired t-test), and after adreno-

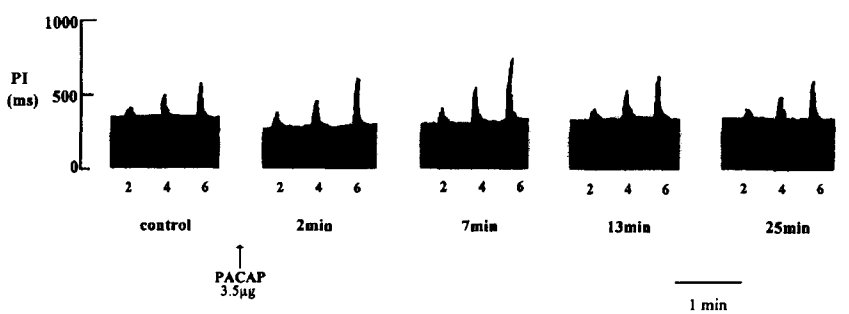

Fig. 2. Original recordings taken from a single experiment illustrate vagal potentiation following PACAP injection. The vertical axis is a measure of pulse interval (PI; ms) and the numbers below each trace indicate the frequency at which the vagus nerve was stimulated. The first panel on the left illustrates vagal slowing at 3 frequencies of vagal stimulation under control conditions while subsequent panels show vagal slowing caused by stimulation at the same frequencies at various times after administration of PACAP. Vagal slowing is potentiated by PACAP 2, 7 and 13 min after injection, but has returned to control levels by 25 min.

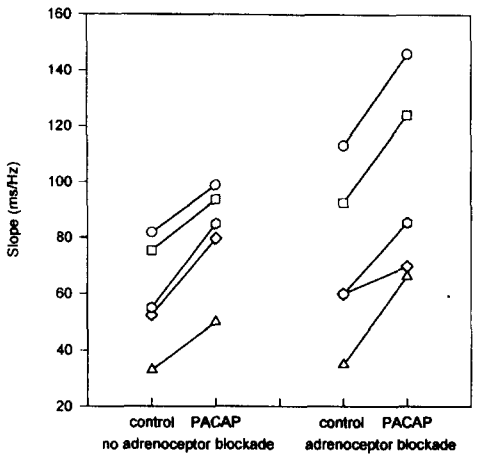

Fig. 3. Potentiation of vagal slowing caused by PACAP is shown in 5 animals, each represented by a different symbol. The vertical axis is a measure of the slope of the regression line (ms/Hz) obtained when vagal slowing in ms was plotted against frequency of vagal stimulation measured in Hz. The slope was recorded under control conditions and after PACAP injection before adrenoceptor blockade on the left of the graph and after adrenoceptor blockade on the right. PACAP causes a similar increase in slope both before and after adrenoceptor blockade.

ceptor blockade was 38 ± 8% (n.s.; $n = 5$). An example from one animal is shown in Fig. 2, where the vagus was stimulated at 2, 4 and 6 Hz: the figure shows that after PACAP 27 administration vagal activity was potentiated and recovery was prolonged. Recovery to control levels of effectiveness for the group was 13 ± 2 min, and this was not affected by adrenoceptor blockade. When 3 frequencies of vagal stimulation were tested, the slope of the regression line of the peak ΔPI for the frequency–response relationship was used. Before PACAP 27 injection, the mean slope of the regression lines was 60 ± 9 ms/Hz and after PACAP 27 was increased to 82 ± 9 ms/Hz ($P < 0.01$; paired t-test), i.e., an increase in slope of 37%. The slopes of the frequency–response relations are summarised for the group in Fig. 3. Adrenoceptor blockade did not significantly change the increase in slope of the frequency–response line caused by PACAP 27 injection.

3.3. Breathing

In all animals breathing increased after PACAP 27 administration. Ventilation was increased to 196 ± 20% of control levels following i.v. injection of the standard dose of PACAP 27 ($P < 0.05$, $n = 7$). Fig. 4 illustrates a typical response from one animal. After adrenoceptor blockade, PACAP 27 injection increased ventilation by the same amount as before

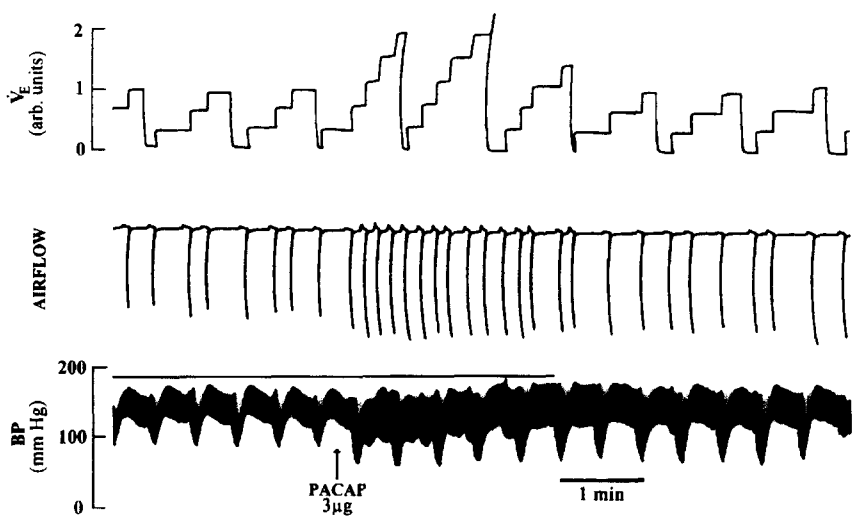

Fig. 4. Original record from a single experiment is shown. The top trace gives a measure of ventilation (V_E in arbitrary units). The middle trace shows the rate of airflow with inspiration shown as the upwards deflection, while the bottom trace is a record of arterial blood pressure (BP; mmHg). When PACAP (3 μg) is given (at arrow), ventilation, rate of airflow and blood pressure are increased. The horizontal line above the blood pressure trace is a reference line.

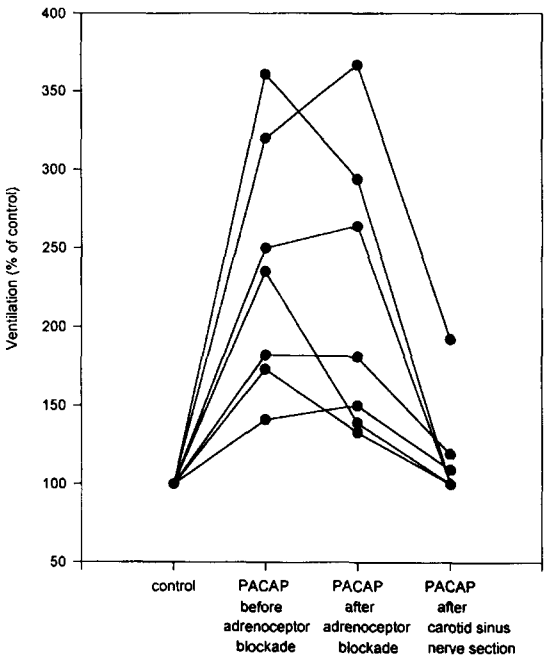

Fig. 5. Effects of PACAP injection on ventilation in 7 animals are illustrated. Control ventilation is recorded as 100%. The effects of PACAP before adrenoceptor blockade, after adrenoceptor blockade and after bilateral section of the carotid sinus nerves are measured as a percent of the control. Each series of filled circles joined by a solid line represents a single animal. PACAP causes a marked increase in ventilation which is not significantly altered by adrenoceptor blockers. However, injection of PACAP given after section of the carotid sinus nerves, did not significantly alter the ventilation when compared to control.

adrenoceptor blockade: $173 \pm 24\%$. However, after carotid sinus nerve section, confirmed by blockade of respiratory response to close intra-arterial injection of CO_2-rich saline, PACAP 27 did not significantly increase breathing ($106 \pm 4\%$ of control). It can be seen (Fig. 4) that the stimulation of breathing lasted 2–3 min, and this was so for each animal. The group data are shown in Fig. 5.

3.4. Sympathetic stimulation

Decreases in pulse interval caused by increasing frequencies of sympathetic nerve stimulation were unaltered by PACAP 27 administration.

Control stimulation of the right cardioaccelerator nerve at 16 Hz in 4 dogs resulted in an inhibition of subsequent cardiac vagal action, as described previously [17]. Mean maximum percentage inhibition was $53 \pm 6\%$ with a time to half-recovery of 10 ± 2 min. After injection of PACAP 27, there was no significant change in the inhibition of vagal action: maximum percent inhibition of vagal cardiodepressor action was $61 \pm 3\%$ with a time to half-recovery of 12 ± 3 min.

4. Discussion

This study has demonstrated significant cardiovascular and respiratory effects of exogenous PACAP 27 in anaesthetised dogs. The novel findings of this study are the dependence of PACAP 27's respiratory stimulation on functional carotid sinus nerves, and the potentiation by PACAP 27 of cardiac vagal action in slowing the heart.

In the present study, PACAP 27 invariably caused an increase in arterial blood pressure which was commonly preceded by a small and transient drop in pressure. This is in keeping with the findings of Suzuki et al. [12] also in anaesthetised dogs, but is in contrast to the work of Ishizuki et al. [11] who found that similar doses of PACAP 27 had a depressor effect and that only much higher doses increased blood pressure. The reason for this discrepancy remains unclear. This study has also shown that the pressor effect of PACAP 27 is greatly attenuated when it is given in the presence of effective adrenoceptor blockade. This finding indicates that at least part of the pressor response is dependent upon catecholamine release and supports the work of Minkes et al. [7], who abolished the pressor effect of PACAP 27 with phentolamine in anaesthetised cats. The same authors also showed the abolition of PACAP 27's pressor effect following adrenalectomy, indicating that PACAP 27 increases blood pressure by stimulating the release of catecholamines from the adrenal gland. However, in contrast to those findings, we found here that a significant part of the pressor effect of PACAP 27 persists after effective adrenoceptor blockade.

In this study, we found that PACAP 27 injection always caused a tachycardia confirming the earlier work of several groups [10–12]. The tachycardia was unaltered by effective β-adrenoceptor blockade indicating that PACAP 27's effect on heart rate is proba-

bly not mediated through release of noradrenaline. It is well known that dibutyryl cyclic AMP, a derivative of cAMP, has positive inotropic and chronotropic actions on the heart [19,20] and since PACAP 27 is a powerful stimulator of cAMP, at least in pituitary cells in vitro [2], it is possible that this may be the mechanism of the tachycardia.

We showed here that PACAP 27 potentiates the cardiac slowing evoked by vagal stimulation. The mechanism of action of this effect was not studied here. PACAP 27 did not affect the ability of cardiac sympathetic nerve stimulation to increase heart rate suggesting no direct effect of this peptide on cardiac sympathetic neuroeffector mechanisms. It also failed to affect the interaction of the sympathetic and parasympathetic nerves supplying the heart, indicating that PACAP 27 has no effect on the release of the vagal-inhibitory cotransmitter (which is probably NPY), from sympathetic nerves [17], nor on the actions of this transmitter on vagal action at the heart. The rationale behind our experiments was to determine whether PACAP 27 stimulated neurotransmitter release from autonomic nerves. We found no evidence for stimulation of release of either noradrenaline or NPY from the cardiac sympathetic nerves. Possibly, therefore, the pressor effect seen after PACAP 27 administration is due to catecholamine release from the adrenal gland as found by Minkes et al. [7]. In contrast with our failure to show effects with PACAP 27 on sympathetic nerves was the potentiation of cardiac vagal action it caused. Possibly this is due to stimulation of acetylcholine release from the vagus: the present study did not investigate the mechanism of this effect.

PACAP 27 has profound effects on breathing. It caused a marked increase in ventilation which was not significantly altered by adrenoceptor blockade but was abolished when the carotid sinus nerves were cut bilaterally. This indicates that the increase in ventilation caused by PACAP 27 is due to stimulation by PACAP 27 of the peripheral chemoreceptors. This has not been previously described.

Acknowledgements

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