Mechanism of action of ATP on canine pulmonary vagal C fibre nerve terminals

Amir Pelleg and Carl M. Hurt

Department of Medicine, Likoff Cardiovascular Institute, Hahnemann University, Philadelphia, PA 19102-1192, USA

1. The effects of extracellular adenosine 5′-triphosphate (ATP) on pulmonary vagal afferent fibres ($n = 46$) was studied in a canine model *in vivo* ($n = 38$).

2. ATP (3–6 μmol kg^{-1}), administered as a rapid bolus into the right atrium, elicited a transient burst of action potentials in cervical vagal fibres, which was not affected by either blockade of ganglionic transmission (hexamethonium) or a drop in arterial blood pressure (nitroglycerine).

3. The fibres with ATP-sensitive terminals were otherwise quiescent with no activity related to either cardiac or respiratory cycles and their conduction velocity was $0{\cdot}85 \pm 0{\cdot}13$ m s^{-1} ($n = 7$).

4. Inflation of the lungs to 2–3 times the tidal volume triggered brief bursts of action potentials in these fibres.

5. Capsaicin (10 μg kg^{-1}), given as a rapid bolus into the right atrium, elicited a burst of action potentials in these ATP-sensitive fibres.

6. Smaller amounts of ATP and capsaicin (0·5–3 μmol kg^{-1} and 1–5 μg kg^{-1}, respectively) had similar effects when the two compounds were given into the right pulmonary artery.

7. Adenosine, adenosine 5′-monophosphate, or adenosine 5′-diphosphate did not excite these fibres ($n = 30$).

8. The non-degradable analogue of ATP α,β-methylene ATP (α,β-mATP) was tenfold more potent than ATP while β,γ-methylene ATP (β,γ-mATP) was inactive.

9. The selective P_{2X}-purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid markedly attenuated the effect of ATP but not of capsaicin. The P_{2Y}-purinoceptor antagonist Reactive Blue 2 was without effect.

10. Pretreatment with pertussis toxin (PTX) did not affect this action of ATP.

11. In the canine lungs ATP activates vagal C fibre nerve terminals. This action is mediated by P_{2X}-purinoceptors and is independent of a PTX-sensitive guanine nucleotide binding protein (G protein).

Extracellular adenosine 5′-triphosphate (ATP) exerts pronounced negative chronotropic and dromotropic effects on sinus node automaticity and atrio-ventricular (AV) nodal conduction, respectively, in feline, canine and human hearts (Belhassen & Pelleg, 1984). A role of the vagus nerve in these actions of ATP was suggested (Emmelin & Feldberg, 1948; Cardenas, Aceves & Alarcon, 1965), and was later confirmed in a series of studies using *in vivo* canine models. These latter studies showed that the depressant actions of ATP on sinus and AV nodes have two components: a neural (i.e. vagal) component and a biochemical component, i.e. adenosine, the product of the rapid enzymatic degradation of ATP (Pelleg, Belhassen, Ilia & Laniado, 1985*a*). Indeed, several perturbations known to modulate parasympathetic input to the heart, e.g. atropine, physostigmine, Ca^{2+} and verapamil, differentially modulated the negative chronotropic and dromotropic actions of ATP and adenosine (Pelleg *et al.* 1985*a*; Pelleg, Mitamura & Michelson, 1985*b*; Pelleg & Michelson, 1987; Pelleg & Hurt, 1990). In addition, prostaglandins, the synthesis and release of which are triggered by ATP, were not involved in the differential potency of ATP and adenosine in the canine heart (Pelleg, Mitamura, Michelson & Dreifus, 1986). The mechanism of the neural component of the action of ATP is not known. We have hypothesized that the enhanced parasympathetic

input to the heart caused by ATP is due to a depressor reflex triggered by the action of the nucleotide on chemosensitive vagal nerve terminals in the heart and/or the lungs (Pelleg & Michelson, 1987), and have recently shown that ATP can symmetrically stimulate vagal afferents in the right and left lungs (Hurt, Wang, Xu, Sterious & Pelleg, 1994). Thus the present studies were aimed specifically at: (1) determining the type of afferent fibres whose terminals are stimulated by ATP in the lungs; (2) identifying the receptor type which mediates this action of ATP; (3) determining whether a pertussis toxin (PTX)-sensitive G protein is involved in the signal transduction of ATP at this site; and (4) determining the role of baroreflex and ganglionic transmission in the afferent traffic elicited by ATP. The present data indicate that extracellular ATP can elicit vagal afferent traffic by activating P_{2X}-purinoceptors located on C fibre terminals in the lungs. Current studies are underway to determine the role of this reflex in pulmonary pathophysiological conditions associated with the release of endogenous ATP into the extracellular fluids.

METHODS

The experimental protocol was approved by the Hahnemann University Animal Care and Use Committee, and is in agreement with the NIH guidelines for the care and use of laboratory animals.

Experiments were performed on anaesthetized (sodium pentobarbitone, 30 mg kg^{-1} plus 3 mg kg^{-1} h^{-1}, I.V.) dogs (17·0 ± 0·6 kg; either sex) artificially ventilated with room air using a respirator. The physiological range for arterial blood pH, P_{O_2} and P_{CO_2} (7·32–7·42, 85–110 mmHg, and 28–41 mmHg, respectively) was maintained by adjustment of the respirator rate and tidal volume as well as by supplemented O_2. Body temperature was maintained with a heating mattress (rectal temperature range, 36·2–37·2 °C). Systemic arterial blood pressure was determined with a Millar pressure transducer located in the descending aorta. A peripheral vein was cannulated for the administration of a physiological saline solution and maintenance doses of the anaesthetic. Catheters were introduced via the right femoral vein and left atrial appendage and positioned in the right atrium and left atrium, respectively, for the administration of test solutions. For intrapulmonary administration of drugs and test compounds, a Swan–Ganz catheter was introduced via a femoral vein and positioned in the distal portion of the right pulmonary artery. The chest was opened by a longitudinal sternotomy. The right cervical vagosympathetic trunk was exposed by a midcervical longitudinal section of the skin and careful dissection of neck muscles and connective tissues. The edges of the cut skin were elevated and secured to create a trough which was filled with warm (37 °C) mineral oil. A section of the vagosympathetic trunk was placed on a small plate of black Perspex and fine branches were separated from the main bundle by careful dissection using microsurgical tools and a dissecting microscope (Model F212, Jenoptik Jena, GmbH, Germany).

Extracellular neural action potentials were recorded using a custom-made bipolar electrode, which consisted of two platinum–iridium wires (1·25 × 0·0125 cm), connected to a high-impedance first-stage differential amplifier (model AC831, CWE Inc., Ardmore, PA, USA) via a shielded cable. The output of the first-stage amplifier was fed into a second-stage differential amplifier (model BMA-831/C, CWE Inc.). Isolated fibres were laid on the pair of platinum wires. Vagal C fibres with chemosensitive endings have a sparse irregular discharge which is never associated with cardiac or respiratory cycles. Confirmation of fibre type was obtained by: first, monitoring the response to capsaicin (10 μg kg^{-1}, intra-right atrial bolus); second, monitoring the response to mechanical stimulation of the lungs using gentle probing with forceps as well as inflation of the lungs to 2–3 times the tidal volume; and third, determining the speed of conduction using a stimulating electrode positioned distal to the initial recording site (Coleridge, Coleridge & Luck, 1965; Coleridge & Coleridge, 1977).

A subgroup of animals was treated with PTX (30 μg kg^{-1}, given into a peripheral vein of conscious animals) 48 h prior to experimentation. (PTX was donated by Dr E. Hewlett, University of Virginia, Charlottesville, VA, USA.) Animals did not show any signs of distress during that period. These animals were subjected to glucose tolerance tests prior to and 48 h after PTX administration to confirm PTX intoxication (Ui, 1984). The test consisted of the application of a bolus of dextrose (1·1 g kg^{-1}, I.V.) and subsequent withdrawal of blood samples (5 ml, every 10 min). Glucose and insulin levels in the blood samples withdrawn during these tests were determined by the Diagnostic Laboratory at Cornell University, New York State College of Veterinary Medicine, Ithaca, NY, USA.

Purine compounds (ATP, adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP) and adenosine; 3–6 μmol kg^{-1}) and capsaicin (10 μg kg^{-1}) were given as a rapid bolus into the right atrium (5 ml test solution + 5 ml physiological saline flush) or the right pulmonary artery (1 ml test solution + 3 ml physiological saline flush). When given in the latter site, smaller doses were used, i.e. 0·5–3 μmol kg^{-1} for purine compounds and 1–5 μg kg^{-1} for capsaicin. α,β-Methylene ATP (α,β-mATP) and β,γ-methylene ATP (β,γ-mATP) were given as one low dose only (0·75 μmol kg^{-1}) to avoid systemic side effects. Volume controls consisted of either 5 + 5 ml or 1 + 3 ml physiological saline. All injections were performed in the same mode by the same person. To exclude involvement of baroreceptors in the recorded neural activity, the latter was monitored before and after a bolus of nitroglycerine (1 mg, I.V.; $n = 5$). The effect of ganglionic transmission blockade on the effects of ATP and capsaicin was determined by the administration of hexamethonium (10 mg kg^{-1}, I.V.; $n = 7$).

To determine the purinoceptor subtype which mediates the action of ATP on pulmonary vagal nerve terminals, ATP and capsaicin were given prior to (control) and following the administration of either the selective P_{2X}-purinoceptor antagonist pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; 15 mg kg^{-1}, I.V.; 2·5–5·0 mg kg^{-1}, intrapulmonary artery; $n = 6$) or the selective P_{2Y}-purinoceptor antagonist, Reactive Blue 2 (RB2, Cibacron Blue 3GA, Sigma; 7·7 mg kg^{-1}, I.V.; $n = 4$) (Burnstock, 1989; Lambrecht *et al.* 1992).

At the end of the experiments animals were killed using sodium pentobarbitone (100 mg kg^{-1}, I.V.) plus 3 M KCl (10 ml, I.V.)

Results are expressed as means ± S.E.M.

Table 1. Effects of capsaicin and ATP on sinus cycle length

	Sinus cycle length (ms)			
	Before	After	Percentage change	*P*
Control				
Capsaicin	485 ± 29	740 ± 80	53 ± 14	0·05
ATP	511 ± 30	1753 ± 172	249 ± 35	0·05
Hexamethonium				
Capsaicin	530 ± 27	530 ± 27	0*	n.s.
ATP	533 ± 25	857 ± 61	61 ± 9*	0·05
PTX				
Capsaicin	328 ± 20	342 ± 26	4 ± 1*	n.s.
ATP	342 ± 30	445 ± 50	29 ± 4*	0·01

Capsaicin (10 μg kg^{-1}) and ATP (6 μmol kg^{-1}) were both given as a rapid bolus into the right atrium under baseline conditions (Control; $n = 13$), following hexamethonium administration (10 mg kg^{-1}, I.V.; $n = 7$) or 48 h following PTX administration (30 μg kg^{-1}, I.V.; $n = 7$). Values are means ± S.E.M.; * $P < 0{\cdot}05$ *vs.* control. n.s., not significant.

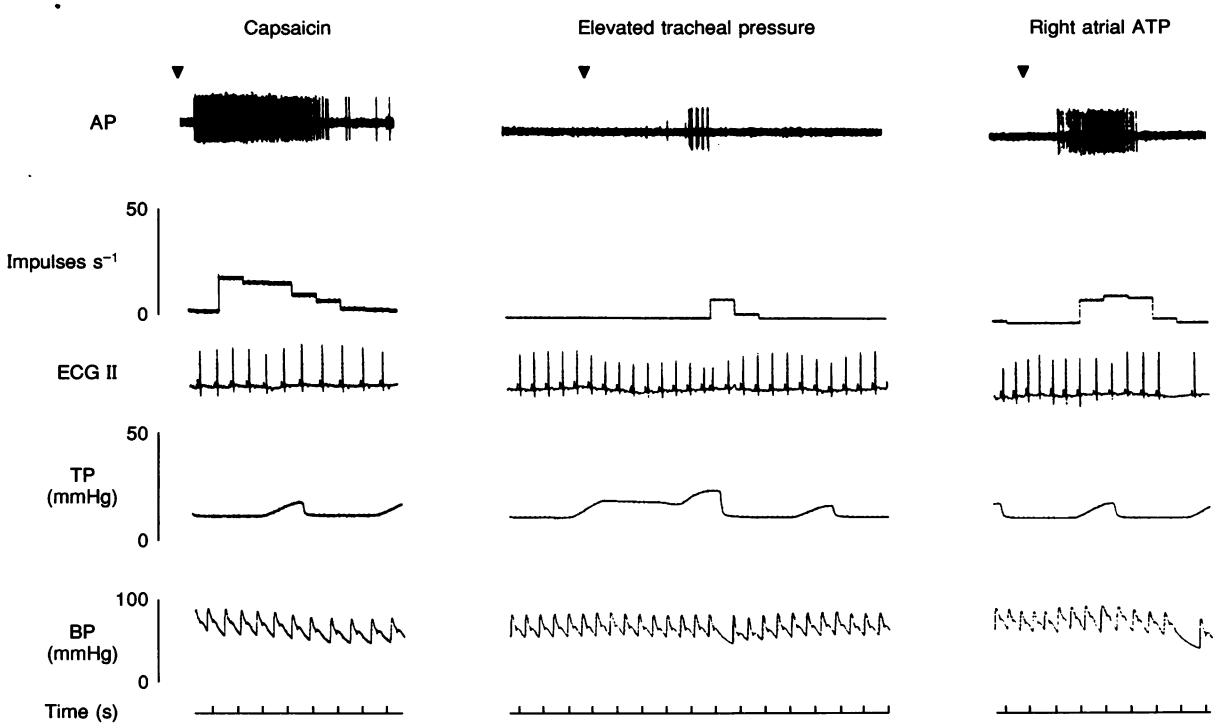

Figure 1. A typical example of the effects of capsaicin, ATP and elevated tracheal pressure in anaesthetized dogs

Capsaicin (10 μg kg^{-1}) and ATP (3 μmol kg^{-1}) were both given as a rapid bolus into the right atrium. Traces from top to bottom: neural electrogram (AP), neural impulses per second, standard lead II electrocardiogram (ECG II), tracheal pressure (TP), systemic arterial blood pressure (BP) and time (s). Left panel: about 1 s following the administration of capsaicin (arrowhead), a transient (8 s) burst of action potentials was recorded from the vagal C fibres. This was associated with mild bradycardia and a slight reduction in systemic arterial blood pressure. Middle panel: the same fibres that were excited by capsaicin were also excited by gradual pulmonary stretch and elevated tracheal pressure caused by a threefold increase in tidal volume (commenced at time indicated by the arrowhead). Right panel: ATP (applied at time indicated by the arrowhead) triggered a transient burst of action potentials in the same fibres that were stimulated by capsaicin as well as by elevated tracheal pressure.

Table 2. Temporal patterns (s) of the actions of capsaicin and ATP

	t_B	D_B	t_{SCL}	t_{BP}
Control				
Capsaicin	$3 \pm 0{\cdot}2$	$6 \pm 0{\cdot}6$	$7 \pm 0{\cdot}5$	$12 \pm 1{\cdot}0$
ATP	$3 \pm 0{\cdot}4$	$4 \pm 0{\cdot}6$	$9 \pm 1{\cdot}0$	$20 \pm 1{\cdot}0$*
Hexamethonium				
Capsaicin	$3 \pm 0{\cdot}1$	$4 \pm 0{\cdot}7$	—	—
ATP	$2 \pm 0{\cdot}1$	$3 \pm 0{\cdot}4$	$13 \pm 0{\cdot}9$*	23 ± 2*

Capsaicin (10 μg kg^{-1}) and ATP (6 μmol kg^{-1}) were given as described in the legend to Table 1. t_B, time to burst of neural action potentials in C fibres; D_B, duration of burst of neural action potentials; t_{SCL}, time to maximal prolongation of sinus cycle length; t_{BP}, time to minimal systemic arterial blood pressure. Values are means ± S.E.M.; * $P < 0{\cdot}05$ *vs.* control t_{SCL}.

RESULTS

In anaesthetized dogs with a stable sinus rhythm, a sinus cycle length of 443 ± 25 ms and mean systemic arterial blood pressure of 89 ± 6 mmHg, the right atrial administration of capsaicin (10 μg kg^{-1}) induced a burst of action potentials in right cervical vagal fibres, which was associated with transient slowing of the heart rate and a drop in systemic arterial blood pressure (Fig. 1). Similar responses were recorded in forty-six fibres in thirty-eight dogs. The administration of ATP caused similar effects, i.e. a burst of action potentials in the same fibres (Fig. 1), a transient prolongation of sinus cycle length (Table 1) and a drop in systemic arterial blood pressure (i.e. capsaicin and ATP maximally reduced blood pressure by 20 ± 4 and 58 ± 3%, respectively). The time-to-peak negative chronotropic effect of ATP was significantly shorter than the time-to-peak vasodilatory effect (Table 2). The elapsed times from the moment of injection of capsaicin and ATP to the beginning of the neural bursts and the duration of the elicited bursts were similar (Table 2). Much smaller amounts of ATP (0·5–3 μmol kg^{-1}) and capsaicin (1–5 μg kg^{-1}) given into the right pulmonary artery elicited similar neural activity to that observed following right atrial administration of these compounds. The

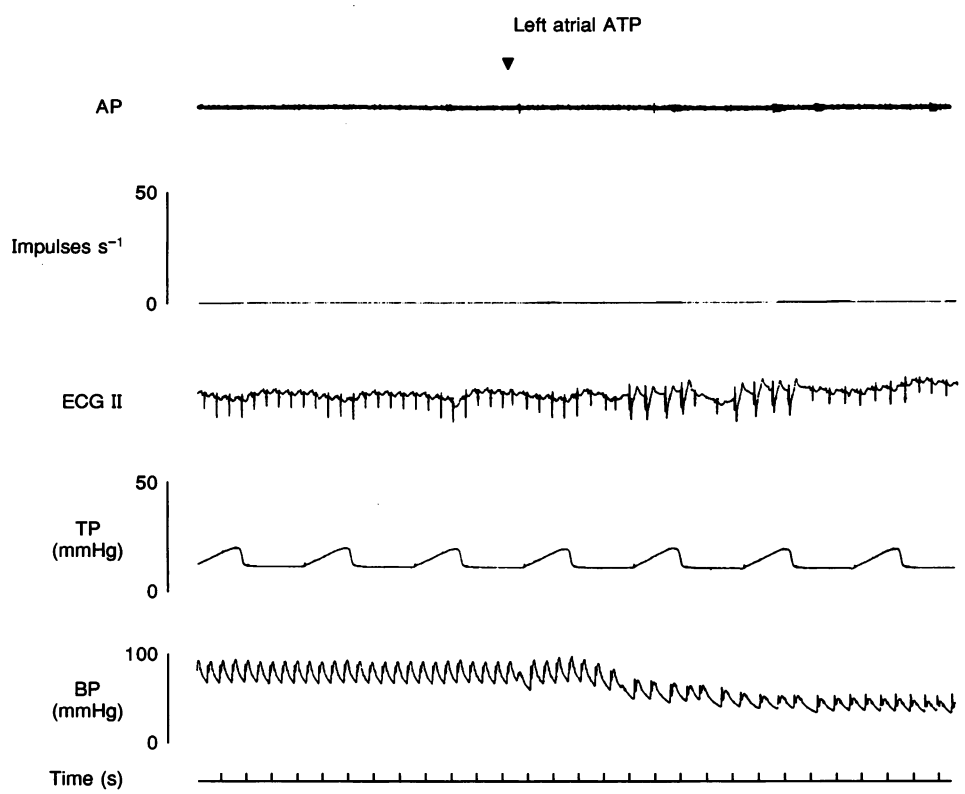

Figure 2. A typical example of the effect of intra-left atrial ATP

Intra-left atrial administration of ATP (6 μmol kg^{-1}; arrowhead) failed to elicit neural activity in right pulmonary vagal afferent C fibres. These fibres were stimulated by intra-right atrial administration of ATP. Traces as in Fig. 1.

degradation products of ATP, ADP, AMP and adenosine, did not excite the fibres ($n = 30$) that were excited by ATP and capsaicin (not shown).

Characterization of the nerve fibres excited by ATP (Coleridge & Coleridge, 1977)

(1) The fibres were otherwise quiescent with no activity associated with either heart rate or respiration cycle. (2) Mechanical stimulation of the right lung elicited a burst of action potentials in the fibres. (3) Elevation of tracheal pressure due to lung inflation to 2–3 times its tidal volume excited the fibres (Fig. 1). (4) Intra-left atrial administration of ATP failed to excite the fibres indicating that their terminals were not in the heart and/or the bronchi (Fig. 2). (5) The time to burst of neural action potentials following right atrial ATP and capsaicin (latency) was short, about 3 s, thus excluding activation of bronchial fibres. (6) The conduction velocity of the fibres was slow ($0{\cdot}85 \pm 0{\cdot}13$ m s^{-1}; range, 0·54–1·58; $n = 7$), within the range of velocities established for canine pulmonary C fibres (Fig. 3).

Effect of nitroglycerine

The drop in systolic arterial blood pressure produced by nitroglycerine (1 mg, I.V.), from 104 ± 12 to 77 ± 8 mmHg (26 ± 6%; $P < 0{\cdot}05$; $n = 5$), did not elicit any specific activity in the fibres in which bursts of action potentials were elicited by capsaicin and ATP.

Effects of hexamethonium

Treatment with the ganglionic blocker hexamethonium (10 mg kg^{-1}, I.V.; $n = 7$) did not alter the afferent traffic elicited by either capsaicin or ATP, but markedly attenuated the negative chronotropic actions of the two compounds (Table 1). The drop in systemic arterial blood pressure caused by capsaicin was also abolished by hexamethonium. In contrast, hexamethonium did not alter the effect of ATP on blood pressure.

ATP signal transduction at pulmonary C fibre terminals

Since neither ADP, AMP nor adenosine (equimolar doses given in the same mode as ATP) induced action potentials in any of the fibres tested, it was hypothesized that P_2-purinoceptors were mediating the action of ATP. To test this hypothesis several pharmacological agents were used.

First, the partially degradable analogue of ATP, β,γ-mATP (0·75 μmol kg^{-1}), did not elicit neural action potentials in any of the fibres studied (5 fibres in 5 dogs). In contrast, α,β-mATP (0·75 μmol kg^{-1}) did elicit bursts of action

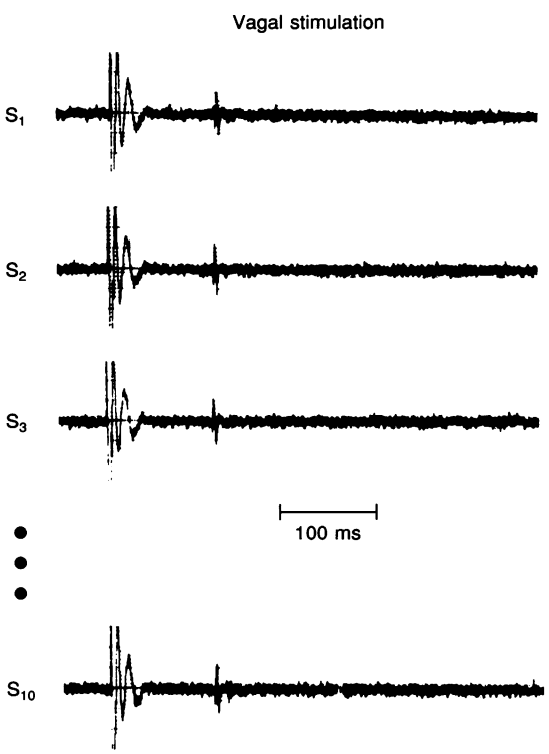

Figure 3. A typical example of traces used for the determination of conduction velocity in capsaicin and ATP-sensitive nerve fibres

The vagus nerve was stimulated in the chest and extracellular neuronal action potentials were recorded in the right cervical vagal fibres sensitive to capsaicin and ATP. S_1, S_2, S_3 and S_{10} denote the first, second, third and tenth vagal stimulus, respectively. The first broad complex signal is a stimulation artifact; the second signal is the conducted neural activity.

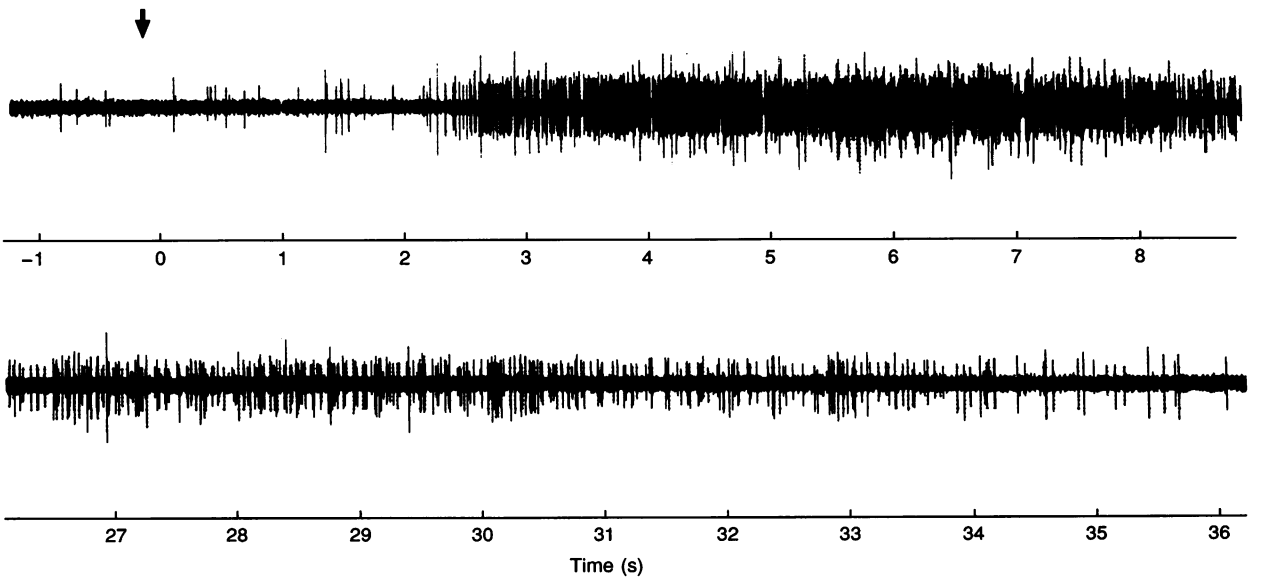

Figure 4. A typical example of neural action potentials elicited in pulmonary afferent C fibres by intra-right atrial α,β-mATP

The arrow indicates the time of administration of α,β-mATP (0·75 μmol kg^{-1}). Time elapsed from the moment of administration is given in seconds. Upper and lower traces are not continuous.

potentials in these fibres ($n = 7$), which were followed by extended periods (2–5 min) of increased activity in comparison with the pre-α,β-mATP conditions (Fig. 4).

Second, the selective P_{2X}-purinoceptor antagonist PPADS markedly attenuated the number of bursts elicited by ATP in pulmonary vagal C fibres ($n = 6$). As can be seen in Fig. 5, intra-right pulmonary administration of PPADS reduced the number of neural action potentials elicited by intra-right pulmonary administration of ATP in a time-dependent manner. In contrast PPADS did not affect the

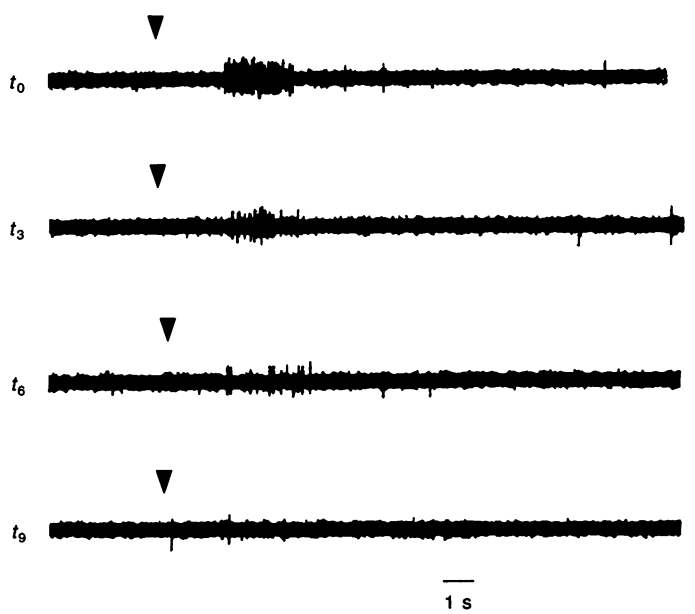

Figure 5. Time-dependent decrease in ATP-elicited bursts of action potentials in vagal pulmonary C fibres following the administration of the P_{2X}-purinoceptor antagonist PPADS

t_0, control trace prior to the administration of PPADS. t_3, t_6 and t_9, traces recorded 3, 6 and 9 min, respectively, following administration of PPADS (15 mg kg^{-1}). Arrowheads indicate the time of ATP (6 μmol kg^{-1}) administration.

Table 3. Plasma insulin and glucose levels before and during glucose tolerance tests

	Insulin (10^{-6} i.u. ml^{-1})		Glucose (mg dl^{-1})	
Time	Control	PTX	Control	PTX
t_0	7 ± 4	53 ± 34	94 ± 13	103 ± 15
t_{10}	60 ± 16	356 ± 44	356 ± 72	346 ± 61
t_{20}	67 ± 41	257 ± 72	230 ± 70	194 ± 31
t_{30}	41 ± 20	185 ± 94	158 ± 51	115 ± 24
t_{40}	24 ± 14	164 ± 119	123 ± 25	96 ± 32
t_{60}	6 ± 3	142 ± 129	91 ± 8	93 ± 37
t_{90}	10 ± 5	141 ± 130	88 ± 10	107 ± 34
t_{120}	8 ± 4	140 ± 130	91 ± 9	125 ± 48

t_0 to t_{120} refer to the time (min) that samples were taken during the glucose tolerance test. Tests were performed prior to (Control) and 48 h after PTX (30 μg kg^{-1}) administration. Values are means ± S.E.M.; $n = 3$.

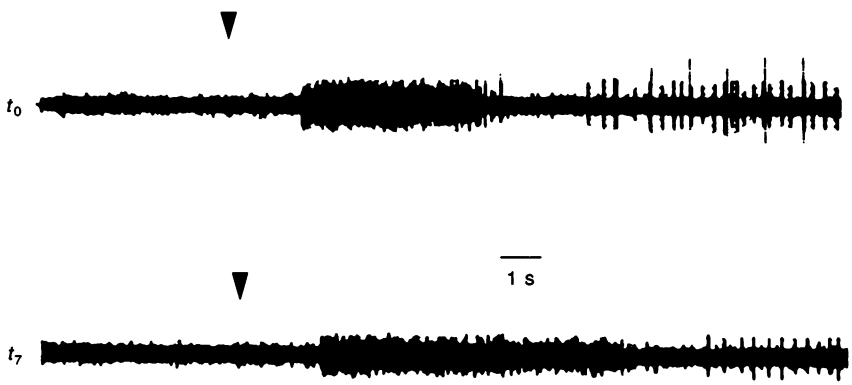

Figure 6. Lack of effect of PPADS, a P_{2X}-purinoceptor antagonist, on the ability of capsaicin to elicit a burst of action potentials in pulmonary vagal afferent C fibres

Top trace, action of capsaicin (10 μg ml^{-1}, given at the time indicated by the arrowheads) prior to administration of PPADS (15 mg kg^{-1}, control, t_0). Lower trace, action of capsaicin administered 7 min after PPADS (t_7).

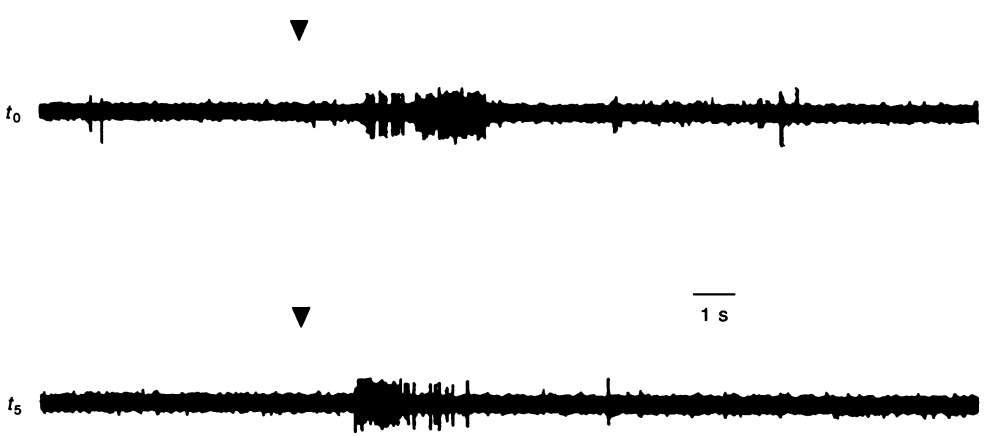

Figure 7. Lack of effect of RB2, a P_{2Y}-purinoceptor antagonist, on the ability of ATP to elicit a burst of action potentials in pulmonary vagal afferent C fibres

Top trace, action of ATP (6 μmol kg^{-1}, given at the time indicated by the arrowheads) prior to RB2 administration (7·7 mg kg^{-1}; control, t_0). Lower trace, action of ATP administered 5 min after RB2 (t_5).

neural response to intra-right pulmonary-applied capsaicin (Fig. 6).

Third, the P_{2Y}-purinoceptor antagonist RB2 did not affect the actions of either ATP (Fig. 7) or capsaicin (not shown) on vagal pulmonary afferent C fibres ($n = 4$).

Effect of PTX

In PTX-treated animals ($n = 7$), there was no change in the neural action potentials evoked by both capsaicin (not shown) and ATP (Fig. 8). In all cases, the two compounds elicited bursts of neural action potentials in vagal afferent fibres (7 fibres in 7 animals). However, the depressant effects on sinus node automaticity were greatly reduced by PTX (Table 1). In contrast, PTX treatment attenuated the reduction in systemic arterial blood pressure caused by ATP to a much smaller extent (i.e. blood pressure was maximally reduced by 58 ± 3 and $39 \pm 10\%$ before and after PTX treatment, respectively).

Data on plasma insulin and glucose levels during glucose tolerance tests indicated that animals treated with PTX were indeed intoxicated. As can be seen in Table 3, the amount of insulin released during the glucose tolerance test was markedly elevated in PTX-treated animals. In spite of that fact, there were only mild differences in the temporal pattern of plasma glucose levels.

DISCUSSION

The major findings of the present study were: (1) an intra-right atrial bolus of ATP elicited a transient burst of action potentials in cervical vagal fibres; (2) similar activity was elicited by capsaicin, given in the same mode; (3) neural activity was elicited in otherwise quiescent slow-conducting fibres; (4) adenosine, AMP or ADP did not elicite this activity; (5) α,β-mATP was much more potent than ATP while β,γ-mATP was inactive; (6) PPADS but not RB2 abolished this action of ATP; and (7) neither PTX nor hexamethonium prevented this action of ATP.

These findings show, for the first time, that exogenous ATP can stimulate pulmonary vagal afferent C fibre terminals by activating P_{2X}-purinoceptors. This supports our hypothesis that ATP triggers a vagal reflex by acting on vagal nerve terminals in the heart and/or the lungs (Pelleg & Michelson, 1987; Pelleg & Hurt, 1990) and recent findings of symmetrical bilateral vagal afferent traffic generated by ATP in the lungs (Hurt *et al.* 1994). Furthermore, the present data also support our hypothesis that the differential potency of ATP *vs.* adenosine in suppressing sinus node automaticity and AV nodal conduction is due to a vagal component in the negative chronotropic and dromotropic actions of ATP, which is absent in the same actions of adenosine (Pelleg *et al.* 1985*a*). Data obtained with hexamethonium are in agreement with this interpretation. The fact that hexamethonium did not alter the transient reduction of blood pressure caused by ATP and the significantly larger time-to-peak effect of ATP on blood pressure *vs.* that of capsaicin indicate that a non-neural factor, i.e. adenosine, the product of the enzymatic degradation of ATP, is mediating, to a large extent, the peripheral vasodilatory action of ATP. In addition, the increase of the time-to-peak effect of ATP on sinus cycle length caused by hexamethonium indicates that

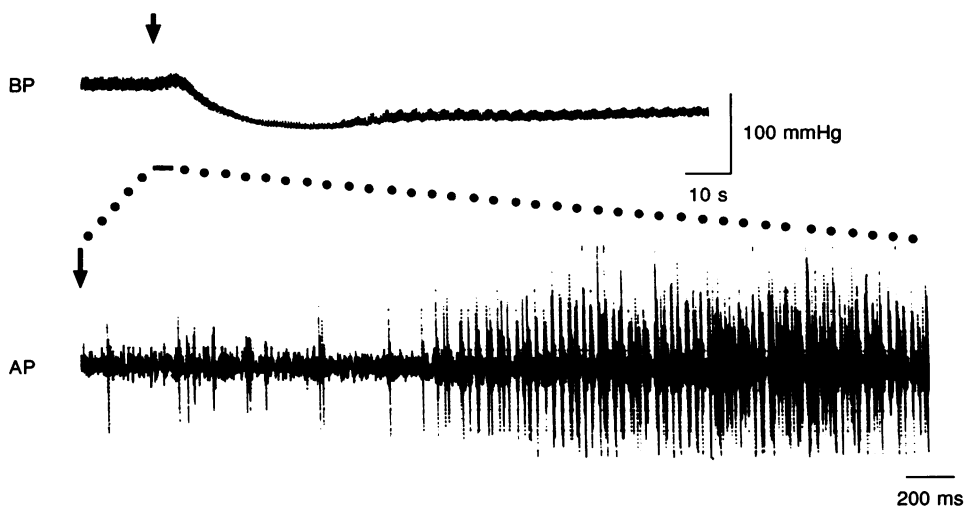

Figure 8. Lack of effect of pretreatment with PTX on the ability of ATP to elicit a burst of action potentials (AP) in vagal pulmonary C fibres and to reduce systemic arterial blood pressure (BP)

ATP (6 μmol kg^{-1}) was given as a bolus into the right atrium. The recording of neural activity in the lower trace (AP) corresponded temporally to the horizontal bar in the upper trace. Note the different time scale for the upper and lower traces. Arrows indicate the time of ATP administration.

the negative chronotropic action of ATP also became dependent on adenosine under experimental conditions in which the depressor reflex was inoperative.

The fact that adenosine, AMP and ADP, unlike ATP, did not elicit neural responses indicates that the action of ATP was mediated by a P_2-purinoceptor. Furthermore, the structure–function cascade: α,β-mATP $\gg$ ATP $\gg$ β,γ-mATP obtained in the present study strongly suggests that the P_2-purinoceptor activated by ATP was of the P_{2X} subtype (Burnstock, 1978). This is supported by the data obtained with the P_2-purinoceptor antagonists. Specifically, PPADS, a P_{2X}-purinoceptor antagonist (Lambrecht *et al.* 1992), but not RB2, a P_{2Y}-purinoceptor antagonist (Burnstock, 1989), effectively blocked the action of ATP on the pulmonary vagal afferent C fibre nerve terminals.

The exact receptor–effector coupling mechanism of the action of ATP is undetermined. To gain insight into ATP signal transduction, a subgroup of animals was treated with PTX. The pronounced potentiation of insulin release during glucose challenge in the PTX-treated animals, and the lack of significant hypoglycaemia under these conditions, are indicative of PTX intoxication. The fact that pre-treatment with PTX did not alter the neural activity elicited by ATP indicates that the action of ATP at the pulmonary vagal nerve terminals is either mediated by a G protein that is insensitive to PTX, or is independent of a G protein. The latter possibility is more likely, resembling signal transduction of ATP in smooth muscle cells and sensory neurons (Bean & Friel, 1990; Benham, 1992). In contrast, PTX attenuated the negative chronotropic action of ATP on sinus node automaticity; this is explained by the role of PTX-sensitive G protein(s) in the signal transduction of both acetylcholine and adenosine, which mediate the action of ATP in the efferent limb of the reflex arc.

Several findings strongly suggest the lungs as the site of action of ATP. First, the action of ATP was similar to that of capsaicin, which is known to act on chemosensitive vagal nerve terminals in the lungs (Coleridge & Coleridge, 1984). Second, the same fibres that were excited by capsaicin and ATP were also excited by pulmonary distension caused by elevated tracheal pressure (2–3 times tidal volume). Third, intra-left atrial ATP failed to excite these fibres. Fourth, adenosine did not mimic the action of ATP on C fibre activity. Finally, mechanical manipulation of the lungs elicited neural activity in all fibres tested.

The actions of capsaicin and ATP were qualitatively similar but quantitatively different. This suggests that the mechanisms of the negative chronotropic and vasodilatory actions on sinus node automaticity and peripheral resistive blood vessels, respectively, of the two compounds are different. In addition, it should be noted that, while ATP and capsaicin stimulate the same afferent fibres, the differential potency and the response of the two compounds to the P_2-purinoceptor antagonist PPADS suggest that different receptors mediate their actions. Furthermore, while all of the capsaicin-sensitive fibres were also sensitive to ATP, the opposite possibility was not tested and, therefore, the existence of ATP-sensitive fibres which are not sensitive to capsiacin cannot be excluded.

Several compounds have been shown to elicit the 'pulmonary depressor chemoreflex' (Dawes & Comroe, 1954). However, most of these are 'foreign chemicals' and only a few are endogenous biological compounds, including the lung autacoids histamine, prostaglandins, bradykinin and serotonin (Coleridge & Coleridge, 1984). The physiological role of the ATP-triggered vagal reflex is still to be determined. However, it is now well established that endogenous ATP can be released into the extracellular space under various physiological and pathophysiological conditions. For example, ATP is released from ischaemic cells (Paddle & Burnstock, 1974; Forrester & Williams, 1977), activated platelets (Mills, Robb & Roberts, 1968) and nerve endings as a cotransmitter (Silinsky & Hubbard, 1973; Burnstock, 1976; von Kugelgen & Starke, 1991). Thus, if the release of ATP occurs in proximity to the ATP-sensitive vagal nerve terminals in the lungs, it is possible that endogenous ATP would trigger an autonomic reflex. This view of ATP as an autacoid interacting with the autonomic nervous system is consistent with the physiological role of ATP in the nervous system, which has been recognized in recent years. Specifically, ATP can depolarize a subset of neurons of various sensory ganglia of rats, cats and bullfrogs (Jahr & Jessell, 1983; Fyffe & Perl, 1984; Krishtal, Marchenko, Obukhov & Volkova, 1988; Bean, 1990; Illes & Norenberg, 1993). In addition, ATP can act as a fast neurotransmitter in the central nervous system (Edwards, Gibb & Colquhoun, 1992) as well as in peripheral ganglia (Fieber & Adams, 1991; Silinsky, Gersanich & Vanner, 1992; Evans, Derkach & Surprenant, 1992). Furthermore, ATP can stimulate chemosensitive afferent nerve terminals (Bleehan, 1978).

ATP is rapidly degraded by ectoenzymes (Ronca-Testoni & Borghini, 1982); however, the present model cannot exclude activation by ATP of vagal afferent terminals in extra-pulmonary tissue, e.g. the heart. It has been shown recently that ATP can modulate canine intrinsic cardiac neuronal activity *in situ* (Huang, Sylven, Pelleg, Smith & Armour, 1993) and stimulate cardiac chemosensitive vagal afferents (Armour, Huang, Pelleg & Sylven, 1994). Thus, it can be hypothesized that ATP given into either the right atrium or the pulmonary artery can stimulate vagal pulmonary afferents and subsequently cardiac afferents. In view of the established asymmetry (i.e. right vagal dominance) in cardiac reflexes (Bishop & Hasser, 1987), this hypothesis could explain the paradoxical findings of asymmetry in

afferent traffic elicited by intra-right atrial ATP (Pelleg, Hurt, Soler-Baillo & Polansky, 1993) *vs.* symmetrical afferent traffic elicited by intrapulmonary ATP (Hurt *et al.* 1994). Therefore, it remains to be determined to what extent vagal afferent traffic elicited in the lungs *vs.* the heart is responsible for the vagal efferent traffic that is manifested by the suppression of sinus node automaticity and atrioventricular nodal conduction.

In summary, the present data have established a mechanism for the pulmonary chemoreflex triggered by the ubiquitous purine nucleotide ATP. This autacoid–neural axis could play an important role in autonomic regulation of pulmonary and cardiac functions under physiological and pathophysiological conditions.

ARMOUR, J. A., HUANG, M. A., PELLEG, A. & SYLVEN, C. (1994). Responsiveness of in situ canine nodose ganglion afferent neurons to epicardial mechanical or chemical stimuli. *Cardiovascular Research* **28**, 1218–1225.

BEAN, B. P. (1990). ATP-activated channels in rat and bullfrog sensory neurons: Concentration dependence and kinetics. *Journal of Neuroscience* **10**, 1–10.

BEAN, B. P. & FRIEL, D. D. (1990). ATP-activated channels in excitable cells. In *Ion Channels*, vol. 2, ed. NARAHASHI, T., pp. 169–203. Plenum Press, New York.

BELHASSEN, B. & PELLEG, A. (1984). Electrophysiologic effects of adenosine triphosphate and adenosine in the mammalian heart: Clinical and experimental aspects. *Journal of the American College of Cardiology* **4**, 414–424.

BENHAM, C. D. (1992). ATP joins the fast lane. *Nature* **359**, 103–104.

BISHOP, V. S. & HASSER, E. M. (1987). Physiological role of ventricular receptors. In *Cardiogenic Reflexes*, ed. MCWILLIAM, P. N. H. & MARY, D. A. S., pp. 62–73. Oxford University Press, Leeds, UK.

BLEEHAN, T. (1978). The effects of adenine nucleotides on cutaneous afferent nerve activity. *British Journal of Pharmacology* **62**, 573–577.

BURNSTOCK, G. (1976). Do some nerve cells release more than one transmitter. *Neuroscience* **1**, 239–248.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: a Multidisciplinary Approach*, ed. STRAUB, R. W. & BOLIS, L., pp. 107–118. Raven Press, New York.

BURNSTOCK, G. (1989). Purine receptors. In *Adenosine Receptors in the Nervous System*, ed. RIBEIRO, J. A., pp. 1–14. Taylor & Francis, London.

CARDENAS, M., ACEVES, J. & ALARCON, G. (1965). Effecto de acido adenosintrifosforico sobre las propiedades fisiologicas del corazon. *Archivos Instituto de Cardiologia de Mexico* **34**, 485–494.

COLERIDGE, H. M. & COLERIDGE, J. C. G. (1977). Impulse activity in afferent vagal C-fibres with endings in the intrapulmonary airways of dogs. *Respiration Physiology* **29**, 125–142.

COLERIDGE, H. M., COLERIDGE, J. C. G. & LUCK, J. C. (1965). Pulmonary afferent fibres of small diameter stimulated by capsaicin and hyperinflation of the lungs. *Journal of Physiology* **179**, 248–262.

COLERIDGE, J. C. G. & COLERIDGE, H. M. (1984). Afferent vagal innervation of the lungs and airways and its functional significance. *Reviews of Physiology Biochemistry and Pharmacology* **99**, 1–40.

DAWES, G. S. & COMROE, J. H. JR (1954). Chemoreflexes from the heart and lungs. *Physiological Reviews* **24**, 167–201.

EDWARDS, F. A., GIBB, A. J. & COLQUHOUN, D. (1992). ATP receptor-mediated synaptic current in the central nervous system. *Nature* **359**, 144–147.

EMMELIN, N. & FELDBERG, W. (1948). Systemic effects of adenosine triphosphate. *British Journal of Pharmacology and Chemotherapy* **3**, 273–284.

EVANS, R. J., DERKACH, V. & SURPRENANT, A. (1992). ATP mediates fast synaptic transmission in mammalian neurons. *Nature* **357**, 503–505.

FIEBER, L. A. & ADAMS, D. J. (1991). Adenosine triphosphate-evoked currents in cultured neurones dissociated from rat parasympathetic cardiac ganglia. *Journal of Physiology* **434**, 239–256.

FORRESTER, T. & WILLIAMS, C. A. (1977). Release of adenosine triphosphate from isolated adult heart cells in response to hypoxia. *Journal of Physiology* **268**, 371–390.

FYFFE, R. E. W. & PERL, E. R. (1984). Is ATP a central synaptic mediator for certain primary afferent fibres from mammalian skin? *Proceedings of the National Academy of Sciences of the USA* **81**, 6890–6893.

HUANG, M. H., SYLVEN, C., PELLEG, A., SMITH, F. M. & ARMOUR, J. A. (1993). Modulation of in situ canine intrinsic cardiac neuronal activity by locally applied adenosine, ATP or their analogs. *American Journal of Physiology* **265**, R914–922.

HURT, C. M., WANG, L., XU, J., STERIOUS, W. & PELLEG, A. (1994). Electrophysiological–anatomical correlates of ATP-triggered vagal reflex in dogs. II. Vagal afferent traffic. *American Journal of Physiology* **267**, H1093–1097.

ILLES, I. & NORENBERG, W. (1993). Neuronal ATP receptors and their mechanism of action. *Trends in Pharmacological Sciences* **14**, 50–54.

JAHR, C. E. & JESSELL, T. M. (1983). ATP excites a subpopulation of rat dorsal horn neurones. *Nature* **304**, 730–734.

KRISHTAL, O. A., MARCHENKO, S. M., OBUKHOV, A. G. & VOLKOVA, T. M. (1988). Receptors for ATP in rat sensory neurons: The structure–junction relationship for ligands. *British Journal of Pharmacology* **95**, 1057–1062.

LAMBRECHT, G., FRIEHE, T., GRIMM, U., WINDSCHEIF, U., BUNGARDT, E., HILDEBRANDT, C., BAUMERT, H. G., SPATZ-KUMBEL, G. & MUTSCHLER, E. (1992). PPADS, a novel functionally selective antagonist of P_2 purinoceptor-mediated responses. *European Journal of Pharmacology* **217**, 217–219.

MILLS, D. C. B., ROBB, J. A. & ROBERTS, G. C. K. (1968). The release of nucleotides, 5-hydroxytryptamine and enzymes from human blood platelets during aggregation. *Journal of Physiology* **195**, 715–729.

PADDLE, B. M. & BURNSTOCK, G. (1974). Release of ATP from perfused heart during coronary vasodilation. *Blood Vessels* **11**, 110–119.

PELLEG, A., BELHASSEN, B., ILIA, R. & LANIADO, S. (1985*a*). Comparative electrophysiologic effects of adenosine triphosphate and adenosine in the canine heart: Influence of atropine, propranolol, vagotomy, dipyridamole and aminophylline. *American Journal of Cardiology* **55**, 571–576.

PELLEG, A. & HURT, C. M. (1990). Evidence for ATP-triggered vagal reflex in the canine heart in vivo. *Annals of the New York Academy of Sciences* **603**, 441–442.

PELLEG, A., HURT, C. M., SOLER-BAILLO, J. M. & POLANSKY, M. (1993). Electrophysiological–anatomical correlates of adenosine 5′-triphosphate-triggered vagal reflex in the dog. *American Journal of Physiology* **265**, H681–690.

PELLEG, A. & MICHELSON, E. L. (1987). Role of the vagus in modulation by Ca^{2+} of the depressant actions of adenosine and adenosine 5′-triphosphate on the canine sinus node in vivo. *Journal of Autonomic Pharmacology* **7**, 127–134.

PELLEG, A., MITAMURA, H. & MICHELSON, E. L. (1985*b*). Evidence for vagal involvement in the electrophysiologic actions of exogenous adenosine and adenosine triphosphate in the canine heart. *Journal of Autonomic Pharmacology* **5**, 207–212.

PELLEG, A., MITAMURA, H., MICHELSON, E. L. & DREIFUS, L. S. (1986). Evidence against prostaglandin mediation of the electrophysiologic effects of ATP versus adenosine in the canine heart. *Journal of Cardiovascular Pharmacology* **8**, 534–538.

RONCA-TESTONI, S. & BORGHINI, F. (1982). Degradation of perfused adenosine compounds up to uric acid in isolated rat heart. *Journal of Molecular and Cellular Cardiology* **14**, 177–180.

SILINSKY, E. M., GERSANICH, V. & VANNER, S. M. (1992). ATP mediates excitatory synaptic transmission in mammalian neurons. *British Journal of Pharmacology* **106**, 762–763.

SILINSKY, E. M. & HUBBARD, J. I. (1973). Release of ATP from rat motor nerve terminals. *Nature* **243**, 404–405.

UI, M. (1984). Islet-activating protein, pertussis toxin: A probe for functions of the inhibitory guanine nucleotide regulatory component of adenylate cyclase. *Trends in Pharmacological Sciences* **5**, 277–279.

VON KUGELGEN, I. & STARKE, K. (1991). Noradrenaline–ATP co-transmission in the sympathetic nervous system. *Trends in Pharmacological Sciences* **12**, 319–324.

Acknowledgements

We thank Drs Drew Armour, Rodrigo Iturriaga, Sukhamay Lahiri and Harold Schultz for their help in introducing the technique of recording afferent vagal traffic into our laboratory and Dr Luiz Belardinelli for helpful suggestions. Pertussis toxin was kindly donated by Dr E. Hewlett. We also thank Drs Li Wang and Jiang Xu for technical assistance and Mrs Dwendolyn Dillard for her secretarial services. A.P. is supported by NIH grant HL43006.

Received 17 March 1995; accepted 11 July 1995.