British Journal of Pharmacology (1996) 119, 1541-1548

P_{2x}-purinoceptors of myenteric neurones from the guinea-pig ileum and their unusual pharmacological properties

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1 Whole-cell and outside-out patch clamp recordings were used to characterize the physiological and pharmacological properties of the P_{2x}-purinoceptors of myenteric neurones from the guinea-pig ileum.

2 Adenosine 5'-triphosphate (ATP) and analogues (1-3000 μM) evoked a rapid inward current in >90% of all recorded neurones. The reversal potential of this current was dependent on the extracellular sodium concentration, at $+14\pm1.9$, 0 ± 1.6 and -12 ± 1 mV for 166, 83 and 42 mM of sodium, respectively. The fast activation and inactivation of this current occurred even when guanosine 5'-triphosphate (GTP) was omitted from the pipette solution or substituted with an equimolar concentration of guanosine 5'-o-[2-thiotriphosphate] (GTP-γ-S). Single channel currents were observed when these outside-out membrane patches were exposed to ATP (10-30 μM). These channels have a unitary conductance of about 17 picosiemens.

3 The rank-order of potency of the agonists used to induce the whole-cell currents was: ATP-γ-S = ATP = 2-methylthio-ATP (2-Me-S-ATP) > > α,β-methylene ATP = β,γ-methylene ATP; adenosine and uridine 5'-triphosphate (UTP) (up to 1 mM) were inactive.

4 Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (1-30 μM) antagonized the effects of ATP (1 mM) with an IC_{50} of 4 μM. α,β-Methylene ATP (100 μM) did not affect the ATP (30 μM)-induced current. Cibacron Blue 3GA increased the ATP activated cationic current whereas Basilen Blue E-3G had a very weak antagonistic effect ($IC_{50} \geq 3$ mM). Suramin potentiated the currents induced by ATP through a mechanism that was independent of its inhibitory effect on ectonucleotidase activity, as suramin also potentiated the effect of α,β-methylene ATP (an ATP analogue that is resistant to nucleotidases).

5 In conclusion, the myenteric P_{2x}-purinoceptor shares some properties with other purinoceptors in particular with the P_{2X_4}- and P_{2X_6}-purinoceptors. This receptor has also some unusual pharmacological properties suggesting that myenteric neurones express a novel subtype of P_{2x}-purinoceptors. The properties of this receptor, however, might be a result of the combination of two or more of the homomeric purinoceptors so far characterized.

Keywords: Enteric neurones; myenteric neurones; ATP receptors; purinoceptors; P_{2x}-purinoceptors; suramin; ligand-gated channels; autonomic neurones; ion channels; pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid

Introduction

Experimental evidence obtained chiefly during the last three decades shows that adenosine 5'-triphosphate (ATP) plays a role as a neurotransmitter and neuromodulator in both central and peripheral synapses. ATP appears to be the principal neurotransmitter in neuromuscular junctions of some arteries, where its release mediates excitatory junction potentials and muscle contraction (Burnstock, 1986; Evans & Surprenant, 1992). In neuronal synapses of the rat habenula (Edwards *et al.*, 1992) and in cultured neurones from the coeliac ganglia (Evans *et al.*, 1992; Silinsky & Gerzanich, 1993), the synaptic release of ATP mediates fast excitatory postsynaptic potentials. These two types of synaptic potentials are mediated by the actions of ATP on P_{2x}-purinoceptors which are ATP-gated ion channels. Such receptors are found in other types of neurones (Bean, 1990; Friel & Bean, 1988; Fieber & Adams, 1991; Barajas-López *et al.*, 1994; Khakh *et al.*, 1995) suggesting that the role of ATP as a fast neurotransmitter is widespread.

Initially, P_{2x}-purinoceptors were defined as having an agonist profile of α,β-methylene ATP > > 2-Me-S-ATP $\geq$ ATP (Bean, 1992; Fredholm *et al.*, 1994; Kennedy & Leff, 1995). However, in some tissues, the potency of some of these agonists is now known to be decreased by their breakdown by ectonucleotidase enzymes, which led in the past to incorrect determination of the agonist potency order (Kennedy & Leff, 1995). After ectonucleotidase inhibition or in studies carried out in single cells, when the ATP catabolism is likely to produce minimal or no change in the ATP concentration, the most common agonist profile of these receptors appears to be 2-Me-S-ATP $\geq$ ATP > α,β-methylene ATP (Kennedy & Leff, 1995). However, different agonist profiles have been obtained in different tissues, suggesting the existence of P_{2x} subtypes (Bean, 1992; Kennedy & Leff, 1995). Such differences are particularly evident with respect to α,β-methylene ATP which is a potent agonist in the ear artery smooth muscle (Benham & Tsien, 1987), and a weaker agonist in vas deferens smooth muscle (Friel, 1988) and in some types of neurones (Fieber & Adams, 1991; Silinsky & Gerzanich, 1993; Barajas-López *et al.*, 1994).

In a previous short communication (Barajas-López *et al.*, 1993), pharmacological evidence was found indicating that myenteric neurones express a different subtype of ATP-gated ion channel. Suramin has been shown to antagonize the actions of ATP on P_2-purinoceptors, including ligand-gated channels in several type of cells (Nakazawa *et al.*, 1990a; Edwards *et al.*, 1992; Evans *et al.*, 1992; Silinsky & Gerzanich, 1993). However, in the myenteric plexus we found that the ATP-evoked currents were unexpectedly increased by suramin

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(Barajas-López *et al.*, 1993). This could be due to a direct effect of suramin on the receptor-channel unit, which would indicate the existence of a novel P_{2x}-purinoceptor in these neurones. Another explanation for such an effect is that suramin inhibits ectonucleotidase activity, as it has been shown recently in other preparations (Hourani & Chown, 1989; Crack *et al.*, 1994). In the present study, we further characterized the physiological and pharmacological properties of P_{2x}-purinoceptors of myenteric neurones to investigate whether they are different from those described for other ATP-gated ion channels.

Methods

Whole-cell currents were recorded from short-term (4–40 hours) primary cultures of myenteric neurones from the guinea-pig ileum. Methods for dissociating and culturing myenteric neurones have been described elsewhere (Barajas-López *et al.*, 1993; 1994). Briefly, the neurones were dissociated using sequential enzymatic treatments, first with a papain solution (0.1 ml ml^{-1}; activated with 0.4 mg ml^{-1} of L-cysteine) and later with a collagenase (1 mg ml^{-1}) plus dispase (4 mg ml^{-1}) solution. After wash out of these enzymes, the cells were plated on rounded coverslips coated with sterile rat tail collagen. Culture medium was minimum essential medium 97.5%, containing 2.5% guinea-pig serum, L-glutamine 2 mM, penicillin 10 u ml^{-1}, streptomycin 10 μg ml^{-1} and glucose 15 mM.

Membrane currents were recorded by an Axopatch 1D amplifier. Patch pipettes were made as previously described (Barajas-López *et al.*, 1994) and had resistances between 1–4 MΩ. With a typical pipette of 2 MΩ resistance and for maximal currents (usually no more than 2–4 nA), the voltage error due to series resistance would be lower than 10 mV, so in most cases, no compensation was made for this factor. The holding potential was usually −70 mV (range −50 to −80 mV). Experiments were performed with the following solution inside the pipette (in mM): Cs-glutamate 160, EGTA 10, HEPES 5, NaCl 10, ATPMg 3 and GTP 0.1; adjusted to pH 7.4 with CsOH. The external solution had the following composition (in mM): NaCl 160, CaCl 2.5, glucose 11, HEPES 5 and CsCl 3; the pH was adjusted to 7.3–7.4 with NaOH. With such solutions the input resistance of the neurones ranged from 1 to 10 GΩ. When ATP concentrations higher than 100 μM were used to stimulate the cells the pH was readjusted with NaOH. Whole-cell currents were recorded with a PC using Axotape software (Axon Instruments). Membrane potentials were corrected for the liquid junction potentials (pipette 11 mV negative). Rapid changes in the external solution were made by using an eight-barrelled device (Barajas-López *et al.*, 1994). ATP and its analogues were applied by abruptly changing the position of the tube, which delivered the external solution, in front of the recorded cell. All experiments were performed at room temperature (~23°C).

The following substances were purchased from Sigma: Basilen Blue E-3G (dye concentration = 60%), Cibacron Blue

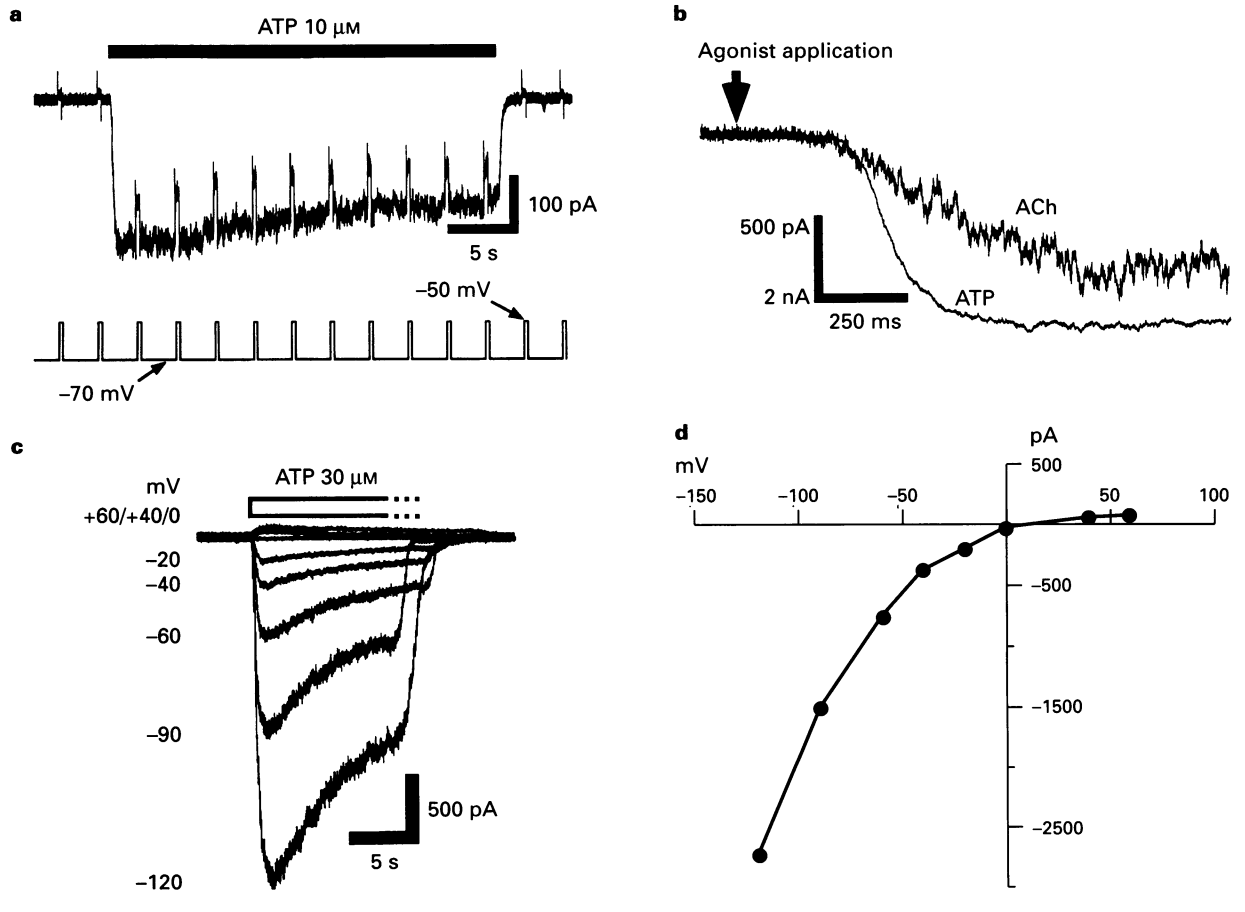

Figure 1 ATP activates a fast whole-cell current which occurs concomitantly with an increase in membrane conductance and shows inward rectification. (a) Upper panel: the time course of the current elicited by ATP in a myenteric neurone. The holding potential was −70 mV and a voltage step to −50 mV (as shown in lower panel) was applied to monitor the membrane conductance. Bars on this and following figures indicate the time of agonist application. Bars were drawn to correspond to the first abrupt detectable deflection of the current. (b) ATP- and ACh-induced (nicotinic) currents have the same latency. Whole-cell currents induced by the application (as indicated by arrow) of either ATP or ACh (100 μM). Note that vertical calibration is 500 pA for ACh and 2 nA for ATP.These recordings were from the same neurone. (c) ATP-induced currents at different membrane potentials and from the same myenteric neurone. (d) Current-voltage relationship from recordings shown in (c).

3GA (dye concentration = 65%), guanosine 5'-o-[2-thiotriphosphate] (GTP-γ-S), adenosine 5'-triphosphate (ATP), α,β-methylene ATP, β,γ-methylene ATP, 2-methylene-ATP (2-Me-S-ATP) and uridine 5'-triphosphate (UTP). The following substances were purchased from Research Biomedical Inc.: acetylcholine (ACh), adenosine and pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS).

Results are expressed as means ± s.e.mean and the number of cells used as *n*. Data from Figures 3 and 7d were fitted to a two-parameter logistic function as previously described (Kenakin, 1993).

Results

ATP application induced a fast inward current (Figure 1) in 94% of (88 out of 94) myenteric neurones. After reaching its maximal amplitude the current decreased slowly in most of the neurones (86 out of 88), for instance, the current decreased to 77 ± 7% ($n = 7$) of its peak amplitude two seconds after the application of 100 μM ATP. However, in two neurones the current decreased very rapidly and it reached a value of less then 10% two minutes after the application of 100 μM ATP. This suggests that a different population of ATP receptors might be expressed in a very low percentage of myenteric neurones. However, this made characterization of these fast responses difficult and these data will not be considered further here. The percentage of neurones of every dissociation that responded to ATP was similar and was also independent of the time (4 to 40 h) that they had been in culture. Concomitant with the ATP-induced current, a significant increase in membrane conductance occurred ($n = 6$; Figure 1a). A latency of 427 ± 11 ms ($n = 5$) was measured between the opening of the tube that was used to apply the agonists (100 μM) and the onset of the current. A similar latency (433 ± 15 ms; $n = 4$) was measured for the nicotinic currents induced by application of ACh (100 μM). Such values were obtained when the tube was placed 500 μM in front of the recorded neurone. When the distance between the application tube and the recorded neurone was decreased to 250 μM, these values decreased to 262 ± 15 and 260 ± 5 ms for ATP ($n = 5$) and ACh ($n = 4$), respectively (Figure 1b), indicating that most of this latency represents the time needed for the ATP and ACh solutions to reach the cell.

The time required to reach the half-maximal current induced by ATP was 105 ± 22 ms for 1 mM ATP ($n = 11$) and its maximal amplitude was usually reached within the next second. ATP current decayed rapidly after wash out of the ATP and was well fitted by a single exponential function ($\tau = 514 \pm 145$ ms). The amplitude of the ATP-induced inward current decreased with depolarization of the membrane and current flowed outward at potentials around +15 mV (Figure 1c). The current-voltage relationships always revealed a strong inward rectification; the most drastic changes in membrane conductance occurred at around −40 mV (Figures 1c and d).

ATP still activated the current when guanosine 5'-triphosphate (GTP) was omitted from the pipette solution (−1109 ± 287 pA; 50 μM; $n = 3$), or when it was substituted with an equimolar concentration of GTP-γ-S

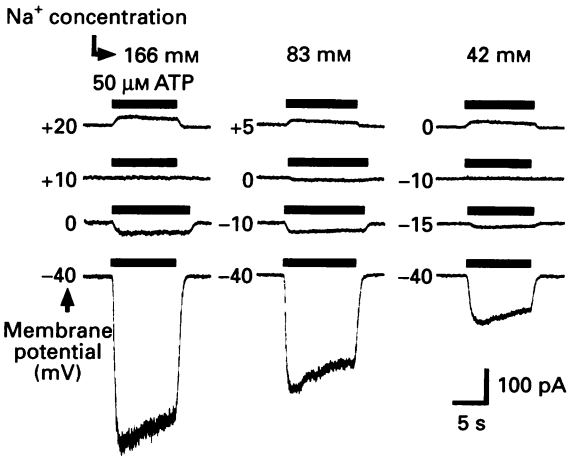

Figure 2 The reversal potential of the ATP-induced current was dependent on the extracellular sodium concentration. Currents were induced by 50 μM ATP at different potentials and in three different concentrations of external sodium (as indicated in mM). Similar observations were obtained in five other myenteric neurones. Sodium ions were replaced by an isosmotic concentration of N-methyl-D-glucamine. All recordings were from the same myenteric neurone.

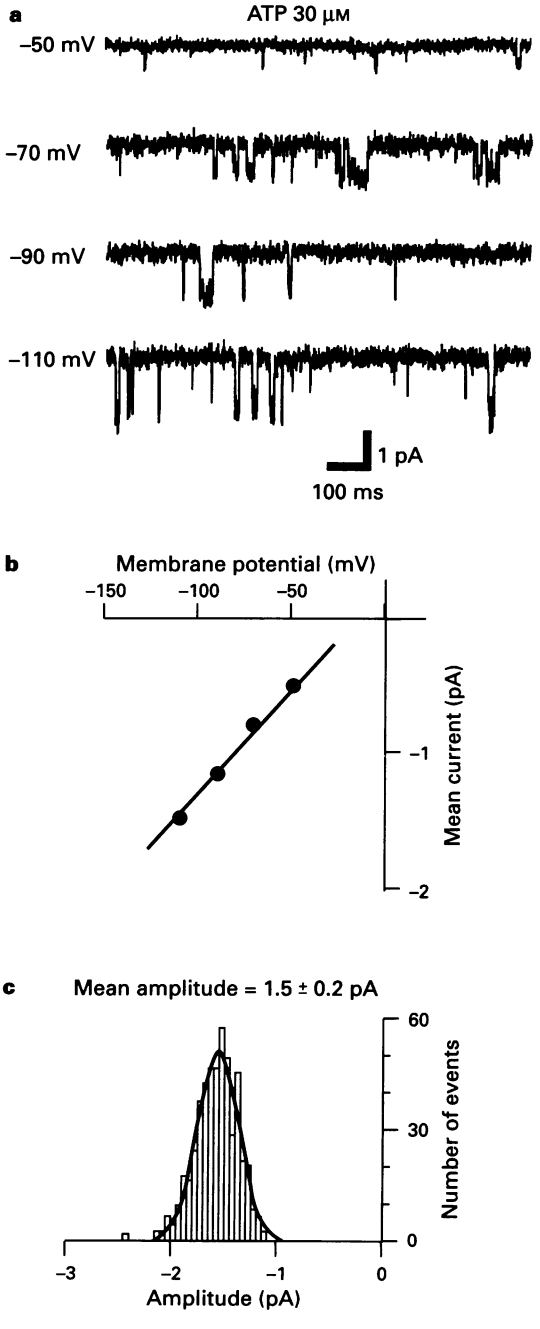

Figure 3 ATP activates single channel activity in membrane patches of myenteric neurones. (a) Amplitude of single channel currents was increased by hyperpolarizing (mV) the membrane patches. (b) Current-voltage relationship of average single-channel activity shown in (a). (c) Amplitude histogram of the same channel at a holding potential of −110 mV. Histogram was obtained from a trace sampled at a frequency of 1 KHz.

(−1182±423 pA; 50 μM; $n=3$). When ATP stimulation was stopped, in the last two experimental situations, the membrane current returned to its control values with a time constant of 546±328 and 430±180 ms, respectively. These findings indicate that G-proteins are not involved in this ATP effect. The reversal potential of this current was dependent on the extracellular sodium concentration, at +14±1.9, 0±1.6 and −12±1 mV for 166, 83 and 42 mM of sodium, respectively (Figure 2; $n=6$). The properties of these ATP-induced currents are very similar to those described previously in other cell types (Bean, 1990; Silinsky & Gerzanich, 1993; Barajas-López *et al.*, 1994; Khakh *et al.*, 1995) and have similar latency to the ACh-evoked (nicotinic) currents, indicating that they are mediated by the opening of ligand-gated channels. These ATP-gated ion channels are, by definition, P_{2x}-purinoceptors (Bean, 1992; Fredholm *et al.*, 1994).

In some cells, an outside-out membrane patch was obtained by pulling away the recording pipette after obtaining the whole-cell configuration. Inward currents, including single channel currents, were observed when these patches were exposed to ATP (10–30 μM). The amplitude of the single channel currents (Figure 3a) or the inward currents of macropatches were increased by hyperpolarization. As shown in the representative recording of Figure 3b, the current-voltage relationship obtained from single channel recordings was linear at potentials more negative than −30 mV. At these potentials an average slope conductance of 17±3 picosiemens was measured ($n=8$; Figure 3b). Similar slope conductance (18 and 20 picosiemens; $n=2$) was recorded when GTP was substituted by an equimolar concentration of GTP-γ-S. In the same patches, evidence for single channel activity was never observed a positive potentials. However, in macropatches small outward currents were noted at positive potentials (12±4 pA; $n=4$). The absence of rectification in the current-voltage relationship of single channels, suggests that the rectification observed in whole-cell experiments (at least for that rectification observed below −40 mV) most likely reflects changes of the single channel open probability. In support of this hypothesis, Silinsky & Gerzanich (1993) observed an increase in the mean open time of ATP-activated single channels by hyperpolarization of patches from neurones of the coeliac ganglia.

Amplitude histograms of single channel activity revealed the existence of one population of channels with an average amplitude of 1.4±0.3 pA at a holding potential of −110 mV ($n=3$, Figure 3c). No subconductance states were observed in the patches tested, which have previously been observed in ATP-activated channels from phaeochromocytoma cells (Nakazawa & Hess, 1993). These observations suggest that whole-cell currents activated by ATP are probably carried out by activation of a single population of P_{2x}-purinoceptors.

Rank order of agonist potency

The amplitude of the inward current was increased when larger concentrations of ATP were used (Figure 4b). Maximal currents were observed at concentrations of 1 or 3 mM of ATP; the mean amplitude of these maximal responses was −2592±198 pA ($n=28$). Concentration-response relationships obtained for several ATP analogues are shown in Figure 4b. The EC_{50} and Hill coefficient for ATP, ATP-γ-S and 2-Me-S-ATP were calculated by the two-parameter logistic function (Kenakin, 1993). Values of the EC_{50} were 55±3, 43±3.4 and 51±4.4 μM, respectively. The Hill coefficients for these three agonists were 1.1±0.09, 1.06±0.08 and 1.11±0.1, respectively, values that were not significantly different from unity. α,β-Methylene ATP and β,γ-methylene ATP were far less potent than ATP in activating the inward current (Figures 4 and 5). In 5 cells that responded to ATP, adenosine or UTP did not induce any change in the holding current at concentrations up to 1 mM (not shown).

Effects of P_2-purinoceptor antagonists

α,β-Methylene ATP has been shown to inhibit strongly ATP actions at P_{2x}-purinoceptors in parasympathetic neurones (Fieber & Adams, 1991). To investigate whether or not the P_{2x}-

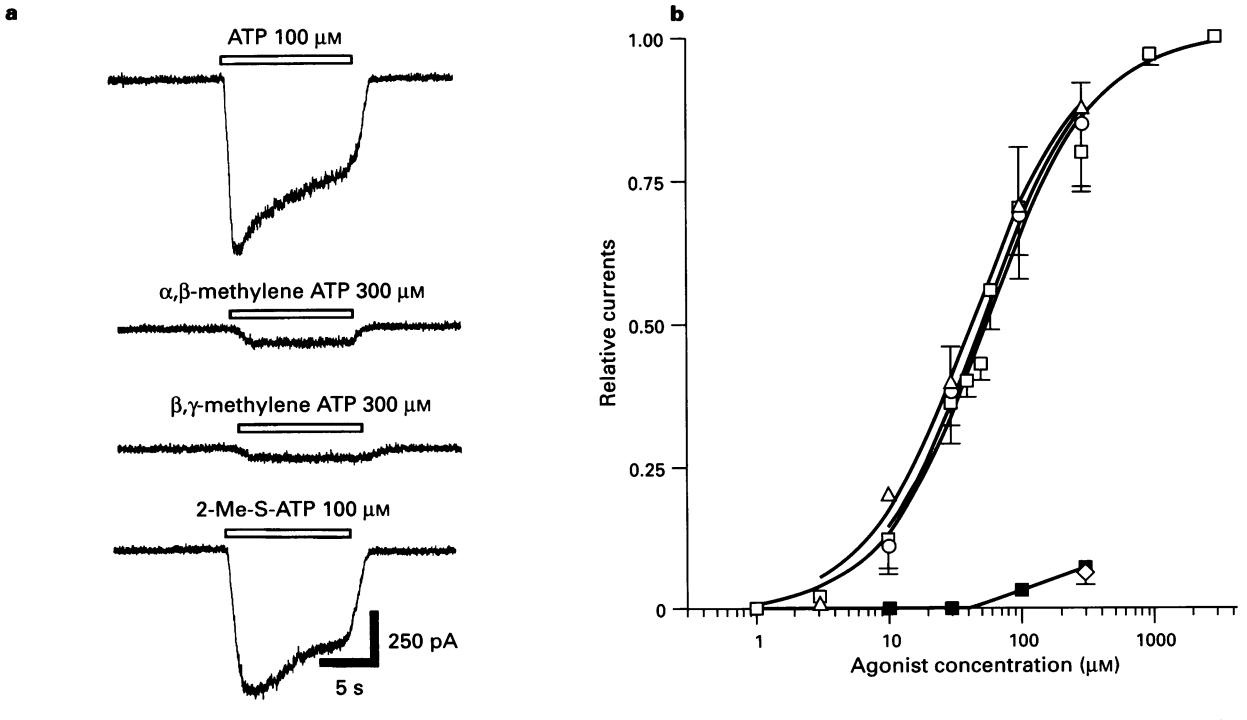

Figure 4 Potency of several ATP analogues to activate the inward current in myenteric neurones. (a) Traces are currents induced by several ATP analogues (as indicated) in the same myenteric neurones. (b) The relative currents, ($I_{agonist}/I_{ATP(maximal)}$) induced by (□) ATP, (○) 2-methylthioATP, (△) ATP-γ-S, (■) α,β-methylene ATP and (◇) β,γ-methylene ATP. Symbols are means and vertical lines show s.e.mean. ($n=4-22$). Sigmoidal lines in this and Figure 7c represent the best fit to a two-parameter logistic function (see Methods).

purinoceptors on the myenteric neurones are similar to those on parasympathetic neurones, we studied the effects of α,β-methylene ATP on the actions of ATP. In myenteric neurones α,β-methylene ATP (100 μM) did not modify the currents induced by 30 mM ATP, whether it was applied during (Figure 5b) or before (Figure 5c) ATP stimulation; currents were -793 ± 75 and -732 ± 110 pA before and during α-β-methylene ATP ($n=4$). The pretreatment with α,β-methylene ATP (100–300 μM) for relatively long periods (10–20 min) did not desensitize ATP effects ($n=6$; Figure 5d); control responses to ATP (30 μM) were 521 ± 68 and 498 ± 75 pA before and 14.3 ± 1.6 min after the start of the superfusion with α,β-methylene ATP.

In myenteric neurones, we had previously noticed that suramin (1–30 μM) increased ATP-induced currents in a concentration-dependent manner and that this potentiation occurred when suramin was applied either before (1–20 min) or during ATP stimulation (Barajas-López *et al.*, 1993). We confirmed these observations in the present study, and to investigate further whether or not this action was due to a known inhibitory effect of suramin on ectoATPase activity (Hourani & Chown, 1989; Crack *et al.*, 1994), we analyzed the actions of suramin (30 μM) on inward currents induced by α,β-methylene ATP (100 μM), known to be highly resistant to nucleotidase activity (Kasakob & Burnstock, 1982; Welford *et al.*, 1987). Suramin always increased the currents induced by α,β-methylene ATP ($n=6$); the average currents were -98 ± 16 pA and -285 ± 29 pA before and in the presence of suramin, respectively (Figure 6). It is important to stress that no evidence of desensitization was observed with this concentration of α,β-methylene ATP. Suramin alone (up to 100 μM) did not change the holding current, which had a value of 30 ± 9 pA in six experiments. Nicotinic currents induced by 30 μM ACh were also not modified by suramin (100 μM), which had an amplitude of 269 ± 117 and 257 ± 122 pA before and in the presence of suramin, respectively ($n=3$; not shown). These observations suggest that suramin acts on a regulatory mechanism specific to ATP-gated channels to potentiate the effects of ATP.

It has been recently shown that PPADS blocks P_2-purinoceptors in several tissues (Lambrecht *et al.*, 1992; Windscheif *et al.*, 1994; Connolly, 1995). In myenteric neurones we found that PPADS (1–30 μM; $n=10$) reduced the amplitude of the ATP-evoked current in a dose-dependent manner (Figure 7a and d). The PPADS concentration required to produce half-maximal inhibition (IC_{50}) of the inward current induced by a maximal concentration of ATP (1 mM) was 4.03 μM. The action of PPADS on the ATP-activated current was measured 3–5 min after the start of its superfusion, when maximal effects were reached. These effects were washed out very slowly; ATP-activated currents were $21 \pm 7\%$ ($n=5$) and $47 \pm 7\%$ ($n=3$) of their control amplitudes 5 and 15 min after stopping PPADS (30 μM) superfusion. PPADS did not affect the inward currents induced by 1 mM ACh, which had a mean value of -896 ± 362 and -937 ± 412 pA before and in the presence of 30 μM PPADS, respectively ($n=3$). Similarly, no change in the whole-cell holding current was induced by PPADS (30 μM).

There are several commercially available Reactive Blue 2 preparations (Burton *et al.*, 1990). Here we tested the effects of two of these dyes, Basilen Blue E-3G and Cibacron Blue 3GA, on the ATP-activated current. Application of Basilen Blue E-3G (30–3000 μM; Figure 7b and d; $n=10$) only partially decreased the currents induced by ATP (1 mM), whereas it did not induce any change in the holding current. The maximal concentration of Basilen Blue E-3G used decreased about 40% of the ATP-increased current (Figure 7d). Nevertheless, the onset of the ATP-induced current was significantly slowed down by Basilen Blue E-3G (Figure 7b); thus, the time required to reach the half-maximal current induced by 1 mM ATP increased by 30 μM Basilen blue E-3G from 127 ± 31 to 561 ± 50 ms ($n=5$). In three other myenteric neurones, it was found that 30 and 300 μM Cibacron Blue 3GA increased the

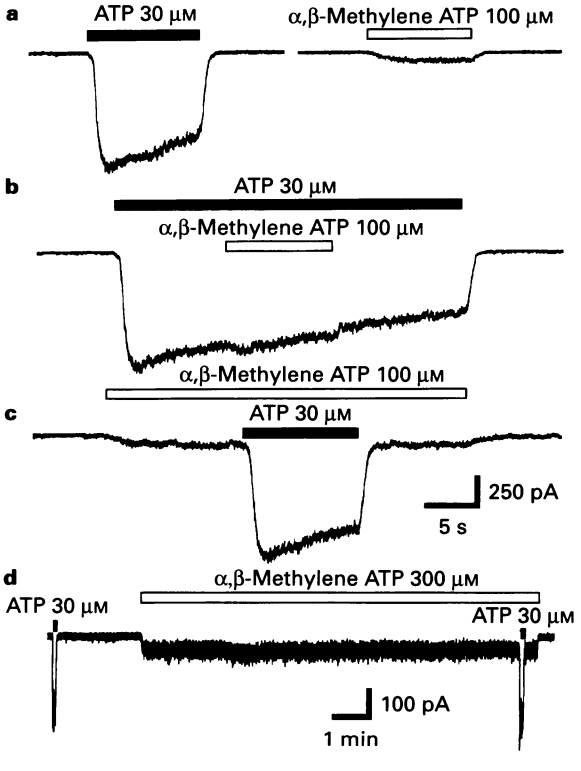

Figure 5 The ATP-induced inward current was not modified by α,β-methylene ATP. (a) Whole-cell currents elicited by ATP and α,β-methylene ATP. α,β-Methylene ATP did not antagonize the ATP action when it was applied either during (b) or before (c) ATP stimulation. Traces shown in (a), (b) and (c) are from the same myenteric neurone and the holding potential was −70 mV. (d) Whole-cell currents elicited by 30 μM ATP before and 10 min after the start of the superfusion with α,β-methylene ATP. Note that α,β-methylene ATP did not desensitize the ATP effect.

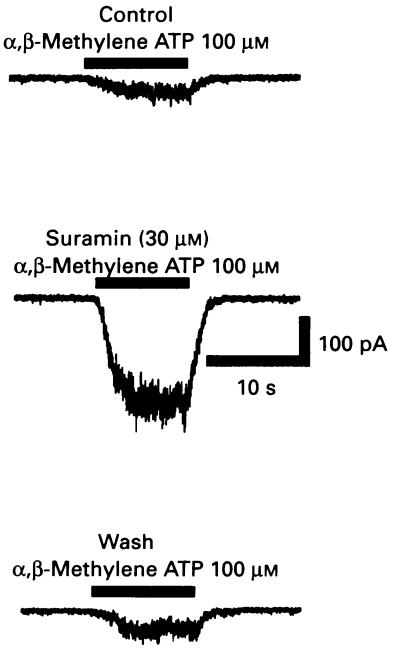

Figure 6 Whole-cell currents elicited by α,β-methylene ATP before, during and 3 min after the washout of suramin (30 μM). Suramin potentiated the currents induced by α,β-methylene ATP. Currents are from the same myenteric neurone and the holding potential was −70 mV.

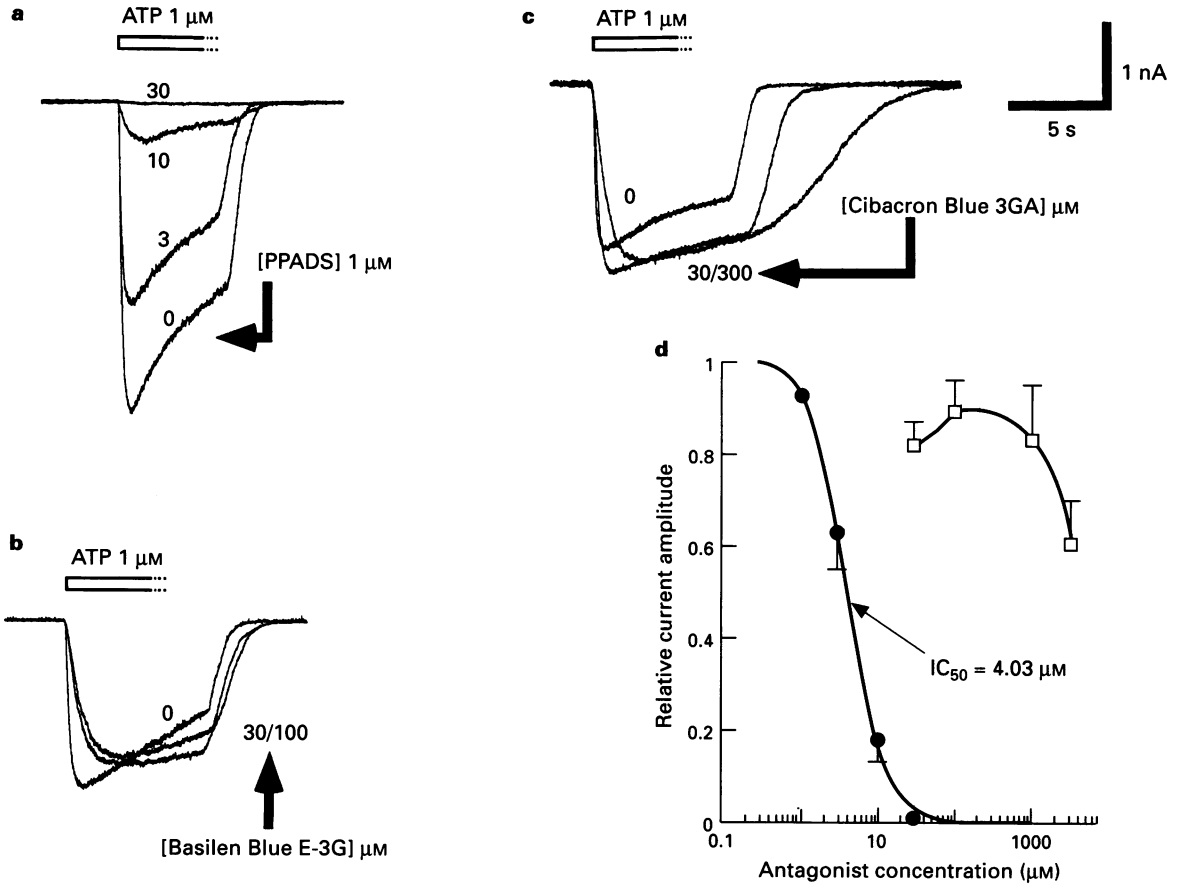

Figure 7 Antagonist profile of the P_{2x}-purinoceptor of myenteric neurones. (a) Whole-cell currents induced by 1 mM ATP before and then in the presence of different concentrations of pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS). (b) Whole-cell currents induced by a maximal concentration of ATP before and then in the presence of 30 and 100 μM Basilen Blue E-3G. (c) Whole-cell currents by 1 mM ATP before and then in the presence of 30 and 300 μM Cibacron Blue 3GA. (d) Concentration-response curves for the effects of PPADS (●) and Basilen Blue E-3G (□) on the inward current induced by ATP. Symbols are means and vertical lines show s.e.mean ($n = 3-5$).

ATP-activated currents by 29±7 and 12±3%, respectively (Figure 7c). The time required to reach half-maximal current induced by 1 mM ATP was also significantly increased by 300 μM Cibacron Blue 3GA, from 236±31 to 669±223 ms ($n = 3$). The effects of these dyes were observed within the first 3 min after application and were fully reversed 10 to 20 min after washout of the dyes.

Discussion

Micromolar concentrations of ATP, but not UTP or adenosine, activate ion channels that are permeable to sodium in myenteric neurones of guinea-pig ileum. Some of the pharmacological properties of this P_{2x}-purinoceptor are different from those of other ATP-gated channels so far described in the literature, suggesting that myenteric neurones express a novel subtype of these receptors. Another explanation is that the different properties of the myenteric P_{2x}-purinoceptor might be the result of the expression and combination of two or more of the homomeric receptors that have been characterized so far.

The existence of a complex population of P_{2x}-purinoceptors was initially suggested based on differences in the agonist and antagonist effects of α,β-methylene ATP (Bean, 1992; Kennedy & Leff, 1995). This ATP analogue behaves as a full agonist in neurones from the nodose and coeliac ganglia (Khakh *et al.*, 1995), whereas it has little or no agonist action in superior cervical neurones (Khakh *et al.*, 1995), parasympathetic neurones (Fieber & Adams, 1991), cardiac atrial myocytes (Friel & Bean, 1988), vas deferens smooth muscle (Friel, 1988), skeletal muscle (Thomas *et al.*, 1991) and in PC12 cells (Nakazawa *et al.*, 1990b). In addition, α,β-methylene ATP has also been shown to antagonize the effect of ATP in superior cervical neurones (Khakh *et al.*, 1995), parasympathetic neurones (Fieber & Adams, 1991) and cardiac atrial myocytes (Friel & Bean, 1988), but not in skeletal muscle (Thomas *et al.*, 1991). In the myenteric neurones (present study) the α,β-methylene ATP was a weak agonist and did not antagonize the actions of ATP on the P_{2x}-purinoceptor, indicating that it has low efficacy and affinity at these receptors.

More recently, six P_{2X} receptor cDNAs have been cloned and their pharmacological properties have been reviewed by Collo *et al.* (1996). One of the major pharmacological differences between these subtypes appears to be in the absolute EC_{50} values for ATP and α,β-methylene ATP. Thus, the P_{2X_1} and P_{2X_3} receptors are activated by high nanomolar to low micromolar concentrations of these two agonists. The other four receptors, P_{2X_2}, P_{2X_4}, P_{2X_5}, and P_{2X_6}, are activated by low micromolar concentrations of ATP but relatively high concentrations of α,β-methylene ATP (> 100 μM). Another striking pharmacological difference is that the ATP effects at P_{2X_1}, P_{2X_2}, P_{2X_3} and P_{2X_5} receptors are antagonized by suramin and PPADS, whereas these two substances have no antagonistic effect at P_{2X_4} and P_{2X_6} receptors. Similar to the latter two receptors, the myenteric P_{2x}-purinoceptor was activated by low micromolar concentrations of ATP and was relatively insensitive to the agonist α,β-methylene ATP. Furthermore, the desensitization of the myenteric P_{2x}-purinoceptors was relatively slow (77%), as in the P_{2X_4} and P_{2X_6} receptors (~62%). There are, however, some significant differences between these three receptors. PPADS, for instance, was an effective antagonist at the myenteric P_{2x}-purinoceptor but has no effect at

the P_{2X_4} or P_{2X_6} receptors (Collo *et al.*, 1996; Buell *et al.*, 1996; Soto *et al.*, 1996). Interestingly, in the latter two receptors, it has been found that the sensitivity to PPADS can be induced by the substitution of a single amino acid (Collo *et al.*, 1996; Buell *et al.*, 1996). Another difference is that ATP-γ-S was a full agonist and as potent as ATP at the myenteric P_{2x}-purinoceptor (present study), whereas it is a partial agonist at the P_{2X_6} (Collo *et al.*, 1996) and less potent than ATP at the P_{2X_4} (Buell *et al.*, 1996). Furthermore, as first demonstrated by our laboratory (Barajas-López *et al.*, 1993), suramin potentiates the actions of ATP at the myenteric P_{2x}-purinoceptor whereas this effect does not appear to be present in the P_{2X_4} or P_{2X_6} receptors (Buell *et al.*, 1996; Collo *et al.*, 1996; Soto *et al.*, 1996). Therefore, it is clear that the myenteric P_{2x}-purinoceptor shares only sone properties with the P_{2X} receptors so far characterized molecularly, in particular with the P_{2X_4} or P_{2X_6} receptors, indicating that it might be a novel subtype of these receptors. Another possibility, however, is that the myenteric P_{2x}-purinoceptor results from the combination of two or more of the six homomeric receptors so far characterized. In agreement with the latter interpretation, it has been proposed that at least some native P_{2X}-purinoceptors could be made up of heteromultimers (Lewis *et al.*, 1995). This interpretation was based on the fact that the receptor (P_{2X_2}) isolated from sensory ganglia has different properties from the native phenotype of these neurones. However, heteropolymerization of these receptors with P_{2X_2} renders ATP receptors with properties similar to those of the neuronal phenotype.

A striking pharmacological property of the myenteric P_{2x}-purinoceptors is that suramin potentiated the ATP effects (Barajas-López *et al.*, 1993) whereas it inhibited ATP actions on P_{2x}-purinoceptors of PC12 cells (Nakazawa *et al.*, 1990a; 1991), central neurones (Edwards *et al.*, 1992; Inoue *et al.*, 1992) and other peripheral neurones (Evans *et al.*, 1992; Silinsky & Gerzanich, 1993; Khakh *et al.*, 1995). This effect of suramin on myenteric neurones was not due to inhibition of ectonucleotidase activity, as suramin also potentiated the effect of α,β-methylene ATP, known to be resistant to nucleotidase activity (Kasakob & Burnstock, 1982; Welford *et al.*, 1987). It was also unlikely to be due to the reactivation of the ATP-desensitized receptors because suramin also potentiated the effects of 3 μM ATP (Barajas-López *et al.*, 1993) or 100 μM α,β-methylene ATP (present study), concentrations at which no evidence of receptor desensitization was observed. One possible explanation for this potentiation is that the myenteric P_{2x}-purinoceptor is formed from subunits with different functional properties, as previously suggested for P_{2x}-purinoceptors (Lewis *et al.*, 1995). Activation of one subunit by ATP might be sufficient to open the channel, which is in agreement with our observation that the ATP Hill coefficient was not different from unity. Activation of a second subunit by ATP might determine allosteric changes in the first subunit and this might decrease its affinity for ATP or modify the electrophysiological properties of the channel, e.g. permeability or kinetics. Suramin could be increasing the currents by antagonizing the effects of ATP on this regulatory subunit. In agreement with this hypothesis, we have shown that higher concentrations of suramin are required to produce this potentiating effect when higher concentrations of ATP are used.

Cibacron Blue 3GA also increased the ATP-activated cationic current in myenteric neurones, whereas Basilen Blue E-3G had a very weak antagonistic effect ($IC_{50} > 3$ mM) on these ATP effects. This further supports the interpretation that the myenteric P_{2x}-purinoceptor is a different subtype. Cibacron Blue 3GA has been shown to block ATP actions on P_{2x}-purinoceptors of neurones (Fieber & Adams, 1991; Silinsky & Gerzanich, 1993; Khakh *et al.*, 1995) and PC12 cells (Nakazawa *et al.*, 1991). Indeed, it has been found that this antagonist has a higher affinity ($K_D = 1$ μM; Fieber & Adams, 1991) than suramin ($K_D = 6.3$ μM; Nutter & Adams, 1994) for P_{2x}-purinoceptors of rat intracardiac neurones; similar observations have been made in PC12 cells (Nakazawa *et al.*, 1991).

PPADS inhibits the fast inward currents induced by ATP in myenteric neurones without affecting the ACh-induced fast inward currents. These results are in agreement with recent studies which showed that PPADS inhibits ATP-induced responses in several tissues (Lambrecht *et al.*, 1992; Connolly, 1995), including responses mediated by P_{2x}-purinoceptors (Valera *et al.*, 1994; Windscheif *et al.*, 1994; Connolly, 1995). In neurones of both enteric plexuses, ATP has also been found to be a potent modulator of synaptic transmission (Kamiji *et al.*, 1994; Barajas-López *et al.*, 1995) and the membrane potassium conductance (Katayama & Morita, 1989; Barajas-López *et al.*, 1994). These effects were seen at ATP concentrations as low as 0.3 μM and are not blocked by PPADS (unpublished observations), indicating that they are mediated by receptors different from the P_{2x}-purinoceptor described here, which is activated at higher concentrations of ATP ($\geqslant 2$ μM) and blocked by PPADS. These observations suggest that PPADS, but not suramin, might be a useful tool to investigate the physiological role of the P_{2x}-purinoceptors and ATP in the myenteric plexus.

Similar P_{2x}-purinoceptors are present in both the myenteric (present study) and submucosal neurones (Barajas-López *et al.*, 1994) of the guinea-pig ileum, as suggested by the fact that the electrophysiological properties and agonist profile of these receptors are virtually the same in neurones from both plexuses. Note that in a previous study (Barajas-López *et al.*, 1994), P_{2x}-purinoceptors of submucosal neurones were misclassified as P_{2y} in agreement with their agonist profile and the previous classification of P_2-purinoceptors (Fredholm *et al.*, 1994; Kennedy & Leff, 1995). P_{2x}-purinoceptors are expressed by both S- and AH-type submucosal neurones (Barajas-López *et al.*, 1994), and probably also by S- and AH-type myenteric neurones. The latter interpretation is supported by our observation that about 90% of all myenteric neurones studied expressed myenteric P_{2x}-purinoceptors, even in experiments carried out a few hours after cell dissociation, and it is known that in the guinea-pig ileum each type of these neurones represents approximately 40–50% of all myenteric neurones (Katayama & Morita, 1989; Surprenant, 1994). It is important to mention that both S- and AH-type neurones (identified electrophysiologically) and Dogiel I and Dogiel II (characterized by microinjection of neurobiotin) have been identified in our cultures for as long as three weeks (unpublished observations), which indicates that both types of neurones can survive in these conditions.

This work was supported by the Medical Research Council of Canada (MT-11642). Carlos Barajas-López was supported by the Ontario Ministry of Health (Career Scientist Award # 04500).

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(Received December 21, 1995
Revised June 21, 1996
Accepted September 16, 1996)