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Colocalization of ATP and nicotinic ACh receptors in the identified vagal preganglionic neurone of rat

Junichi Nabekura, Shinya Ueno*, Tetsuro Ogawa and Norio Akaike*

*Department of Physiology, Akita University, School of Medicine, Akita, 010, Japan and *Department of Physiology, Kyushu University, School of Medicine, Fukuoka, 812, Japan*

1. Effects of exogenous adenosine 5′-triphosphate (ATP) and acetylcholine (ACh) were investigated on acutely dissociated preganglionic neurones in the dorsal motor nucleus of vagus (DMV) of rats using whole-cell patch clamp recording methods.
2. The DMV neurones identified by retrograde transport of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) fixed onto the cervical vagal nerve bundle were large in size (25–35 μm diameter) and bipolar or tripolar in shape.
3. About 90% of DiI labelled DMV neurones responded to both ATP (10^{-4} M) and ACh (10^{-4} M) with inward currents at a holding potential (V_h) of −40 mV.
4. The ATP-induced current (I_{ATP}) and the ACh-induced current (I_{ACh}) reversed their polarities at membrane potentials between +5 and +15 mV, indicating that ATP and ACh increase the membrane permeability to cations.
5. The inhibitory potency of Reactive Blue on 5×10^{-4} M I_{ATP} is more effective (concentration for half-inhibition (IC_{50}), $4{\cdot}4 \times 10^{-7}$ M) than suramin (IC_{50}, $6{\cdot}0 \times 10^{-6}$ M). In addition, α,β-methylene ATP up to 10^{-4} M could not induce any current. As intracellular application of guanosine 5′-*O*-(2-thiodiphosphate) (GDPβS) did not block the I_{ATP}, the I_{ATP} was mediated not by guanosine triphosphate (GTP) binding protein, but rather by ligand-gated ionic channels, presumably via P_{2X} receptors.
6. Currents produced by ACh were due to activation of nicotinic receptors because they were mimicked by nicotine and carbachol, and blocked by hexamethonium. In addition, muscarine evoked no response.
7. Only 25% of nucleus tractus solitarii (NTS) neurones and no hypoglossal neurones responded to the exogenous application of ATP.
8. These results suggest that vagal preganglionic neurones colocalize functionally nicotinic and P_{2X} purinergic receptors.

Extracellular adenosine 5′-triphosphate (ATP) has physiological effects on a variety of tissues; i.e. muscles, neurones, hepatocytes, and mast cells of several mammalian species (Gordon, 1986). For instance, ATP stimulates the Ca^{2+} mobilization in hepatocytes (Charest, Blackmore & Exton, 1985), neurones (Ueno, Harata, Inoue & Akaike, 1992) and megakaryocytes (Uneyama, Uneyama & Akaike, 1993). Extracellular ATP evokes catecholamine release, and its metabolite, adenosine, inhibits the release of transmitters from presynaptic nerve terminals (Stone & Taylor, 1978; Jackisch, Fehr & Hertting, 1985). In addition, ATP has been widely noted to act as a proposed transmitter, or a cotransmitter, with noradrenaline (NA) or acetylcholine (ACh) since Burnstock (1972) provided the purinergic nerve hypothesis. ATP has been released at neuro-neuronal synapses in the nervous system, such as in medial habenula (Edwards, Gibb & Colquhoun, 1992) and coeliac ganglion (Evans, Derkach & Surprenant, 1992) of rats. Locus coeruleus neurones of the rat show an excitatory postsynaptic response to extracellular ATP in slice preparation (Tschopl, Harms, Norenberg & Illes, 1992; Shen & North, 1993). One-third of neurones in the vagal complex of the rat brainstem, including the nucleus tractus solitarii (NTS), exhibit an extracellular ATP-gated cation channel (Ueno *et al.* 1992). In this preparation, ATP caused an excitation through a receptor channel complex, classified as P_{2X} (Abbracchio & Burnstock, 1994).

The vagal complex in the brainstem, comprising the NTS and dorsal motor nucleus of the vagus (DMV), is known to

have a function in autonomic control (Reis, 1984; Loewy & Spyer, 1990). The vagal complex contains neurones with receptors for ATP (Ueno *et al.* 1992) and is composed of a variety of different cell types, including preganglionic neurones, neurones receiving primary afferents from peripheral organs, and those involved with respiration (Loewy & Spyer, 1990). Such functional diversity might suggest a similar diversity of ATP responses in these neurones. To form a functional assessment of the actions of ATP, it is essential to determine its action on different classes of neurone within the vagal complex. Therefore, in the present study, identified single preganglionic neurones were dissociated from the DMV using lipophilic fluorescent dye. The main aim of the present experiments was to examine whether a population of neurones with ATP receptors also possesses nicotinic ACh or NA responses and to elucidate regional and neuronal characteristics in the population of neurones which respond to extracellular ATP in the vagal complex.

METHODS

Identification of the vagal preganglionic neurones in DMV

Thirty-eight Wistar rats (12–16 days old) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} I.P.). One cervical vagal nerve bundle (either right or left) was exposed and a small piece of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes) was fixed onto the nerve bundle. After repairing the incision, the animals were returned to the individual mother's cages. Observing growth and behaviour, the rats which had undergone operation showed no significant difference in general appearance from sham-operated rats and those not operated on. After allowing at least 4 days for the retrograde transport of DiI to the DMV region, brain slices of medulla oblongata were then taken (see below for details). To limit any damage to living tissues caused by fluorescent light when labelled neurones were visualized, exposure of neurones to the fluorescent light was minimized using neutral density filters placed in the light path. For fluorescent excitation, an excitation of 530–560 nm and a low-pass filter of 580 nm were used to view the labelled neurones. Images of neurones stained with DiI were collected with a confocal microscope adapter (VX100, Newport, Irvine, KY, USA) and an intensified CCD camera (ICCD-100, Hamamatsu photonics, Hamamatsu, Japan) attached to an inverted microscope (TMD 300, Nikon). Fluorescent images with a low light level were obtained with an image processor (DVS-1000, Hamamatsu Photonics) and recorded using a DAT tape recorder (Sony).

Cell dissociation

DMV neurones were dissociated as described previously (Nabekura, Ebihara & Akaike, 1993). Briefly, 16- to 21-day-old Wistar rats treated with DiI were decapitated under ether anaesthesia. The brainstems were removed and dissected into coronal slices (350 μm thick) using a microslicer (DTK-1000, DSK, Kyoto, Japan). The slices containing the DMV region were incubated in a standard solution equilibrated with 95% O_2–5% CO_2 at room temperature (23–25 °C) for 50 min. After the preincubation, the slices were treated with enzyme (dispase, 1000 U ml^{-1}) for 60 min at 31 °C. Subsequently, the DMV region was punched out and the neurones were dissociated mechanically. Electrophysiological measurements were applied to the cells with fluorescence within 20 min after dissociation.

Electrical recording

Recordings were carried out using a nystatin-perforated patch recording configuration under whole-cell conditions (Nabekura *et al.* 1993). Both the current and the membrane potential were measured with a patch-clamp amplifier (CEZ-2300, Nihon Kohden, Tokyo, Japan). All signals were filtered with a low-pass filter with a cut-off frequency of 1 kHz, monitored on both a storage oscilloscope (R5133, Tektronix, Beaverton, OR, USA) and a pen recorder (Recti-Horiz-8K, San-ei, Tokyo, Japan), and stored on video tapes after digitizing at a rate of 44 kHz (PCM-501ESN, Sony). The resistance between the recording electrode and the reference electrode was 3–5 MΩ. The cell capacitance and series resistance were 16–32 pF and 3–20 MΩ (70–80% compensated), respectively. All experiments were performed at room temperature (23–25 °C).

Solutions

Dissociated DMV neurones were submerged in the standard perfusate with a constant flow (3–4 ml min^{-1}). The ionic composition of the standard external solution was (mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 Hepes. The pH was adjusted to 7·4 with tris(hydroxymethyl)aminomethane (Tris-base). For nystatin-perforated patch recording, the patch pipette solution contained 150 mM KCl, 10 mM Hepes and 100 mg ml^{-1} nystatin. The pH of internal solutions was adjusted to 7·2 with Tris-base. For conventional whole-cell recording, pipettes were filled with (mM): 150 KCl, 5 $MgCl_2$, 5 Na_2ATP and 10 Hepes (pH 7·2).

Drugs

Drugs used in the experiments were dispase (Godoshusei, Tokyo, Japan), nystatin, Na_2ATP, ACh, 5-hydroxytryptamine (5-HT), Reactive Blue 2 and GDPβS (Sigma) and suramin (a kind gift from Bayer, Wuppertal, Germany). All drugs were dissolved in the external solution just before use. Drugs were applied using a rapid application method termed the 'Y-tube' method, as described previously (Nabekura *et al.* 1993). Using this technique, the solution surrounding a neurone could be exchanged within 20 ms.

Statistical analysis

Experimental values are presented as means ± S.E.M. For evaluation of the half-maximal effective concentration (EC_{50}) and Hill coefficient (n) of the concentration–response curve, data were fitted to the Michaelis–Menten equation using a least-squares fitting:

$$I = I_{max}C^n/(C^n + K_d{}^n), \quad (1)$$

where I is current, I_{max} is maximum response, and C is the concentration of agonist. The equation for the concentration–inhibition curve is the mirror image of the Michaelis–Menten equation:

$$I/I_{max} = 1 - C^n/(C^n + K_d{}^n), \quad (2)$$

where C is the concentration of antagonist. Assuming that the value of I_{max} is 1, then

$$I = 1 - C^n/(C^n + K_d{}^n) \quad (3)$$

and

$$I = K_d{}^n/(C^n + K_d{}^n). \quad (4)$$

The data for the concentration–inhibition curve were fitted to eqn (4) by the use of a least-squares fitting to obtain the half-maximal inhibition concentration (IC_{50}).

RESULTS

Identification of vagal preganglionic neurones in DMV

Serial coronal brain slices (350 μm thick) of the brainstem were obtained from 16- to 21-day-old rats. DiI, placed onto either the right or left cervical vagal nerve trunk was transported retrogradely, to label preganglionic motoneurones in the DMV (Fig. 1*A*). The distribution of DiI-labelled neurones was restricted to the DMV and the ventral area of brainstem (probably in the nucleus ambiguus) ipsilateral to the placement of DiI. After treatment of brain slices with an enzyme, the DMV containing the fluorescence was punched out and the neurones were dissociated mechanically. Neurones stained by DiI and having resting potentials more hyperpolarized than −40 mV in current clamp mode were chosen for further electrophysiological studies. The input resistance measured in current clamp mode was 262 ± 37 MΩ ($n = 7$) DiI-labelled neurones were large in size (25–35 μm diameter) and either bipolar or tripolar in shape (Fig. 1*B* and *C*).

ATP- and ACh-induced currents in DMV neurones

Over 90% of the DiI-labelled DMV neurones tested (48/52) responded to both 10^{-4} M ACh and 10^{-4} M ATP. ATP (10^{-4} M) evoked a peak and sequential gradual decrease of inward current (I_{ATP}) during continuous application of ATP at a holding potential (V_h) of −40 mV (Fig. 2*A*). On the other hand, ACh (10^{-4} M) produced a transient inward current followed by a rapid current decrease (I_{ACh}) (Fig. 2*B*). The peak current amplitudes of I_{ATP} and I_{ACh} increased in a concentration-dependent manner. The threshold for I_{ATP} was around 1 μM. The experimental data for ATP response fitted a theoretical curve with a Hill coefficient of 1·2 and an EC_{50} of 60 μM (Fig. 2*A*). The concentration–response curve for ACh had a Hill coefficient of 1·1 and an EC_{50} of 74 μM (Fig. 2*B*). Two out of fifty-two DMV neurones responded only to ATP and not to ACh, while only a single neurone responded to ACh and not to ATP. One neurone responded to neither ATP nor ACh (Table 1). However, the relationship between the peak amplitude of I_{ATP} (10^{-4} M) and that of I_{ACh} (10^{-4} M) in the neurones which responded both to ATP and ACh varied considerably from cell to cell ($1·43 \pm 0·52$, $n = 25$, ratio of I_{ATP} to I_{ACh} at a V_h of −40 mV).

The reversal potentials for I_{ATP} (E_{ATP}) and I_{ACh} (E_{ACh}) in the standard internal and external solutions estimated from the relationship between V_h and their amplitudes were 15 ± 3 mV ($n = 6$) and 5 ± 3 mV ($n = 5$), respectively, although marked inward rectifications of I_{ATP} and I_{ACh} disturbed the accurate measurement of their data (Fig. 3). To examine the involvement of Cl^- channels in these currents, E_{ATP} and E_{ACh} were obtained in different

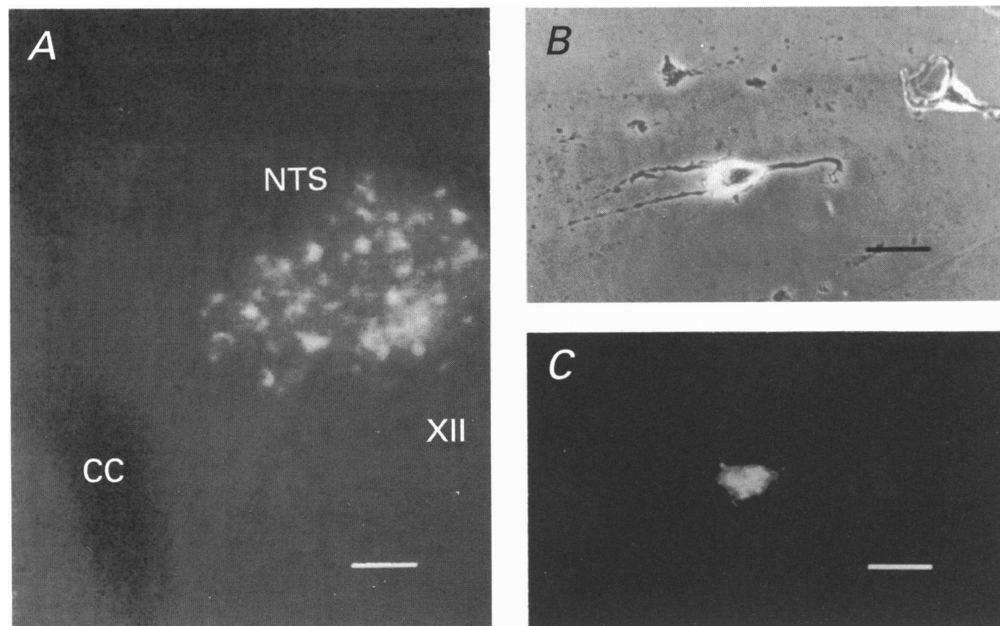

Figure 1. Photographs of dorsal brainstem and acutely dissociated neurones

A, combined picture of transmitted image with dim light and fluorescent image. This photograph shows the coronal section through the dorsal medulla. DiI-staining was restricted to the DMV region. On the other hand, no stained neurones were observed in the nucleus tractus solitarii (NTS) or hypoglossal nucleus (XII). CC, central canal. Scale bar represents 300 μm. *B*, photograph of dissociated neurones under phase-contrast optics. The neurones were photographed with an inverted microscope. *C*, epifluorescent image of neurones presented in *B*. The neurones at the left and right in *B* are stained and not stained by DiI, respectively. Scale bars in *B* and *C* represent 30 μm.

extracellular Cl^- concentrations ($[Cl^-]_o$) because the theoretical equilibrium for Cl^- was −1·8 mV in the standard internal and external solutions. Changing $[Cl^-]_o$ to 71 mM did not affect either E_{ATP} (13 ± 4 mV, $n = 4$) or E_{ACh} (7 ± 6 mV, $n = 4$). There were no significant differences in E_{ATP} or E_{ACh} between the two $[Cl^-]_o$ conditions ($P > 0·05$, Student's two sample t test). This finding suggests that ATP and ACh open cation channels but not Cl^- channels.

Our previous report (Ueno *et al.* 1992) noted that I_{ATP} was mediated by an ion-gated purinergic receptor in vagal complex (mainly NTS) neurones. In the present study, intracellular application of GDPβS (100 μM) in a conventional whole-cell recording did not block the I_{ATP}, where the peak amplitude of I_{ATP} and the ratio of I_{ATP} to I_{ACh} obtained 20 min after rupture of the patch membrane were 322 ± 92 pA ($n = 5$) and 1·12 ± 0·23 ($n = 5$), respectively. This suggests the I_{ATP} is mediated not by a

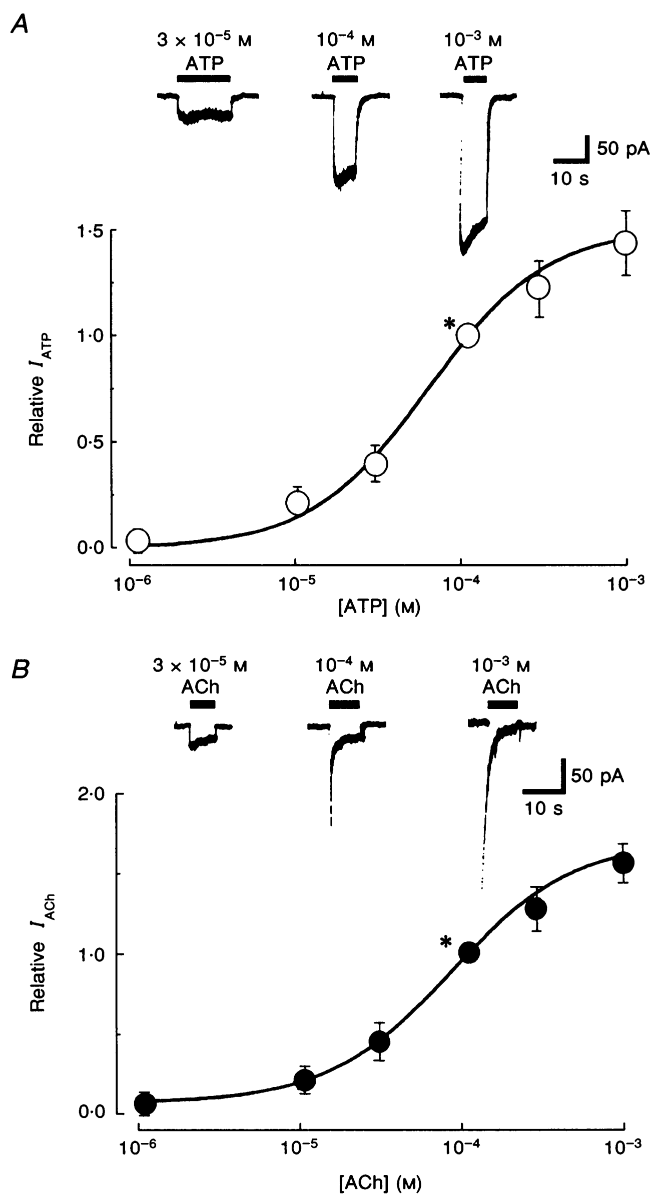

Figure 2. Concentration dependence of ATP- and ACh-induced peak currents

A, concentration–response curve for the ATP-induced currents. DMV neurones were clamped at a V_h of −40 mV, and various concentrations of ATP were applied every 5 min. All peak current amplitudes were normalized to the peak current induced by 100 μM ATP (*). Throughout the figures each symbol and bar represents the mean ± S.E.M.; 7–8 neurones were tested. A continuous curve was fitted to the data using eqn (1) (see Methods). Inset shows actual currents induced by ATP at various concentrations. *B*, concentration–response curve for the ACh-induced currents. All responses were normalized to the peak current obtained with 100 μM ACh (*); 7–8 neurones were tested.

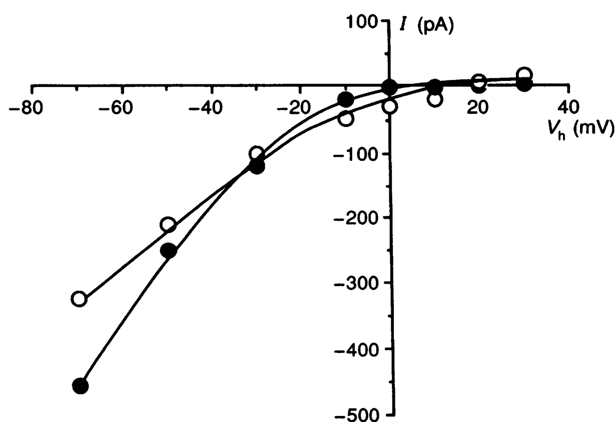

Figure 3. Current–voltage relationships for ATP (○) and ACh (●)-induced peak currents

Each response was evoked at the concentration of 10^{-4} M. Both curves indicate inward rectifications at membrane potentials more depolarized than −20 mV. Both curves were obtained from the same cell.

GTP-binding protein-coupled purinergic receptor, but by ligand-gated ionic channels.

The effects of P_2 antagonists were examined on the I_{ATP} in identified DMV neurones. Suramin, the most commonly used non-selective P_2 antagonist, inhibited I_{ATP} in a concentration-dependent manner, in which IC_{50} was 6 μM (Fig. 4*A* and *B*). Reactive Blue 2 also suppressed I_{ATP} in a concentration-dependent manner. Reactive Blue 2 was effective at concentrations greater than 10^{-8} M and the IC_{50} was 4.4×10^{-7} M. The inhibitory potency of Reactive Blue 2 on the I_{ATP} was 10 times greater than that of suramin. On the other hand, α,β-methylene ATP (10^{-4} M, $n = 6$) did not produce any current (Fig. 4*C*).

The I_{ACh} was completely suppressed by hexamethonium (100 μM), but not by 1 μM Reactive Blue 2 (94·3 ± 6·4% of control, $n = 4$) or 100 μM suramin (93·7 ± 6·3% of control,

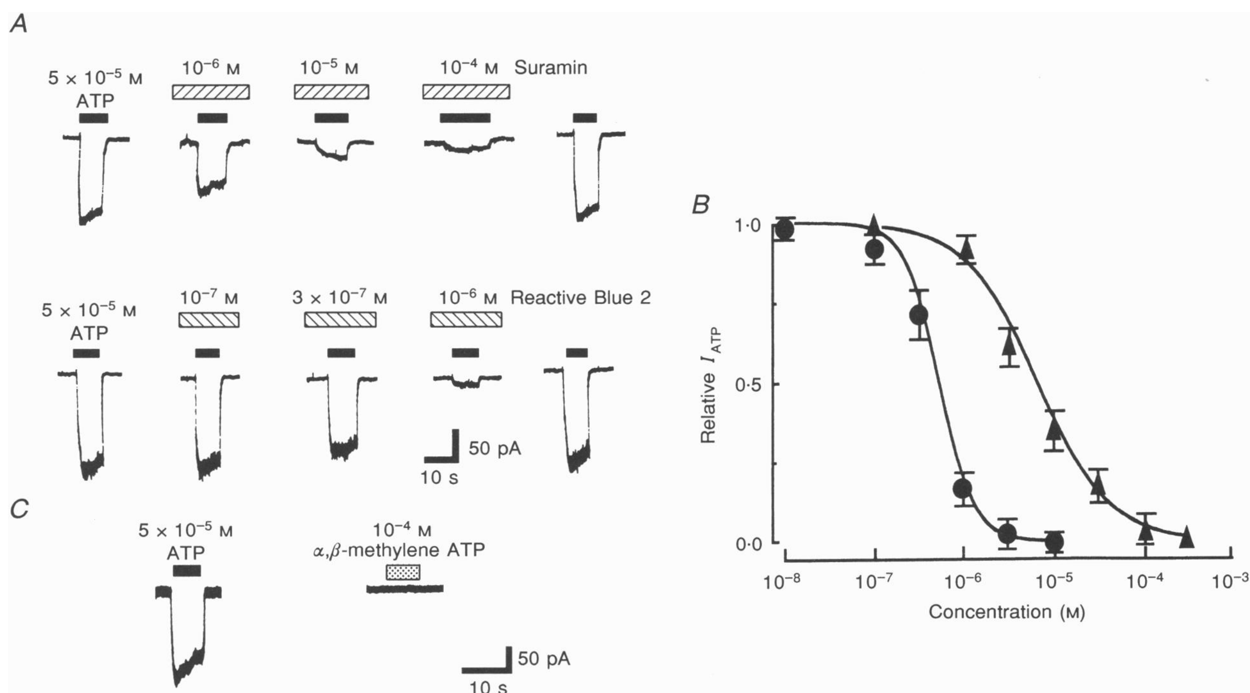

Figure 4. Effects of purinergic antagonists on ATP-induced current

A, control response was obtained with the application of 5×10^{-5} M ATP. Neurones were pretreated with suramin or Reactive Blue 2 for 1 min before application of ATP. *B*, concentration–response relationships for inhibition of ATP-induced currents by suramin (▲) and Reactive Blue 2 (●). The curves were fitted to the data using eqn (4) (see Methods); 8–9 neurones tested. *C*, no effect of α,β-methylene ATP (10^{-4} M) on ATP-responding neurone. Holding potential, −40 mV.

Table 1. Colocalization of ATP and ACh responses

	DMV neurones responding		NTS neurones responding	
	With ATP	Without ATP	With ATP	Without ATP
With nACh	48 (92·3 %)	1 (1·9 %)	10 (15·9 %)	8 (12·7 %)
Without nACh	2 (3·8 %)	1 (1·9 %)	6 (9·5 %)	39 (61·9 %)

Total number of DMV neurones tested, 52; total number of NTS neurones tested, 63. Responses are to 10^{-4} M ATP and nACh (nicotinic acetylcholine). Results are given as a percentage of the total in parentheses.

$n = 4$). The application of nicotine (100 μM) and carbachol (100 μM) could mimic the I_{ACh} in all ACh-responsive DMV neurones tested ($n = 5$), whereas muscarine (100 μM) did not induce any current at V_h of -40 mV ($n = 20$). Hexamethonium (100 μM) suppressed I_{ATP} only by $3{\cdot}5 \pm 3{\cdot}1$ % of control ($n = 7$).

In peripheral ganglion neurones, ATP, ACh and 5-HT also operate cation channels (Bean, 1990; Evans *et al.* 1992). However, neither 5-HT (10^{-5} M, $n = 16$) nor NA (10^{-5} M, $n = 12$) induced any current in the DMV neurones in this study.

Regional difference

The application of ATP evoked an inward current in about 96 % of DiI-labelled DMV neurones tested (50/52). Of the cells showing an ATP response, 96 % (48/50) were also responsive to ACh (10^{-4} M) with inward currents. In contrast, no recordings obtained from neurones dissociated from the hypoglossal nucleus, which is adjacent to the DMV (Fig. 1*A*), exhibited any response to ATP ($n = 18$). Twenty-five per cent (16/63) and 29 % (18/63) of NTS neurones responded to ATP and ACh, respectively. Only 16 % (10/63) of NTS neurones responded to both ATP and ACh. The proportion of NTS neurones responding to both ACh and ATP was much lower than the proportion of DiI-labelled DMV neurones showing ATP and ACh sensitivity (Table 1). In addition, to examine whether DiI labelling induced the ACh and ATP responses, the effects of ATP (10^{-4} M) and ACh (10^{-4} M) were examined on labelled substantia nigra neurones stained by the injection of 20 % DiI solution (in dimethyl sulphoxide) into the caudate nucleus. Only one out of twelve and two out of thirteen labelled substantia nigra neurones responded to ATP and ACh, respectively. These findings indicated the existence of both ATP and ACh responses in almost all DMV neurones.

DISCUSSION

This study demonstrated that about 90 % of vagal preganglionic neurones identified with DiI responded to both extracellular ATP and ACh, which activated P_{2X} and nicotinic receptors, respectively.

With regard to peripheral nervous preparations, ATP-gated cation channels exist in rat sensory neurones (Krishtal, Marchenko & Pidoplichko, 1983), bullfrog dorsal root ganglion neurones (Bean, 1990), rat parasympathetic cardiac ganglion cells (Fieber & Adams, 1991) and guinea-pig coeliac ganglion neurones (Evans *et al.* 1992). In these preparations, extracellular ATP activates the ligand-gated channels defined as P_{2X} receptors. In central neurones such as NTS (Ueno *et al.* 1992), locus coeruleus (Shen & North, 1993) and medial habenula neurones (Edwards *et al.* 1992), ATP also causes an inward current mediated by ligand-gated cation channels. On the other hand, the GTP-binding protein coupled with a purinergic receptor (Burnstock & Kennedy, 1985; Boyer, Cooper & Harden, 1990) is classified as a P_{2Y} receptor (Abbracchio & Burnstock, 1994). GDPβS in the patch pipette blocked the GTP-binding protein-mediated muscarinic ACh response within 5 min after rupture of patch membrane in our conventional whole-cell recording (Nabekura *et al.* 1993). Using the same recording condition, intracellular application of GDPβS did not block the I_{ATP} for 20 min in the present study, indicating the participation of the P_{2X} receptor in the I_{ATP}. In addition, because α,β-methylene ATP is less effective on the DMV (Fig. 4), the I_{ATP} might be mediated by P_{2X4} or other P_{2X} receptors which are less sensitive to α,β-methylene ATP (Nakazawa, Fujimori, Takanaka & Inoue, 1990; Abbracchio & Burnstock, 1994; Brake, Wagenbach & Julius, 1994).

Electrophysiological studies showed that the autonomic ganglion cells and rat PC12 cells possess nicotinic ACh, 5-HT_3 and ATP receptors (Higashi & Nishi, 1982; Nakazawa *et al.* 1990; Furukawa, Akaike, Onodera & Kogure, 1992). On the other hand, the present experiments showed that the dissociated DMV neurones responded to both ATP and ACh but not to 5-HT. This result could be accounted for by the report that 5-HT_3 receptors are located only on the presynaptic nerve ending of vagal afferents (Waeber, Dixon, Hoyer & Palacios, 1988; Pratt & Bowery, 1989; Leslie, Reynolds, Andrews, Grahame-Smith, Davis & Harvey, 1990) or by the distal distribution of 5-HT receptor in the DMV neurones because acute

Table 2. Excitatory action of ATP in rat CNS neurones

Reference	Neurones	ATP response (%)	nACh response (%)
Current paper	DMV	96	94
	Hypoglossal nucleus	None	—
	NTS	25	29
	Substantia nigra	8	15
Ueno *et al.* (1992)	Vagal complex	34	—
Shen & North (1993)	LC	100	—
	Mesencephalic nucleus	None	—
	Parabrachial nucleus	None	—
Edwards *et al.* (1992)	Medial habenula	100*	+
Tschopl *et al.* (1992)	LC	83	—

DMV, dorsal motor nucleus of vagus; NTS, nucleus tractus solitarii; LC, locus coeruleus; None, no response; +, existence of neurones with ATP and ACh responses but no statement of the number; *, response to α,β-methylene ATP.

dissociation can maintain only soma and proximal processes (Fig. 1*B*).

In the central nervous system (CNS), ATP-activated cation channels have been reported in the DMV, NTS, locus coeruleus and medial habenular regions (Edwards *et al.* 1992; Ueno *et al.* 1992; Shen & North, 1993). Considering the functional characteristics of these regions, some common features may exist. The DMV is the major source of vagal parasympathetic fibres that innervate mainly abdominal visceral organs as well as cervical and thoracic organs (Kalia, 1981). The DMV neurones respond electrophysiologically to various proposed transmitters such as somatostatin (Nabekura, Mizuno & Oomura, 1989), amino acids (Travagli, Gillis, Rossiter & Vicini, 1991), thyrotropin-releasing hormone (Travagli, Gillis & Vicini, 1992) and ACh (Ito, Fukuda, Nabekura & Oomura, 1989). In addition, most DMV and habenular neurones are parts of cholinergic pathways (Simon, Oderfeld-Nowak, Felten & Aprison, 1981). Referring to the purinergic peripheral system, it might be worth investigating the noradrenergic and cholinergic pathways in the CNS. Indeed, almost all locus coeruleus neurones, which are catecholaminergic, respond to ATP (Shen & North, 1993). This nucleus projects to the DMV, NTS and habenular regions (Gottesfeld, 1983; Ross, Ruggiero & Reis, 1985; Ter Horst, Toes & Van Willigen, 1991). The ascending NTS pathways transmit a wide range of visceral information to hypothalamus, forebrain and catecholamine synthesizing neurones such as the ventrolateral medulla, locus coeruleus and raphe nucleus (Ross *et al.* 1985). Reciprocally, these CNS regions send efferents to NTS (Sofroniew, 1983; van der Kooy, Koda, McGinty, Gerfen & Bloom, 1984; Ross *et al.* 1985). These nuclei with ATP-sensitive neurones have dense anatomical connections to each other and are commonly involved in the autonomic functions and in cholinergic or noradrenergic pathways.

At some cholinergic and noradrenergic terminals of peripheral neurones, especially those related to the parasympathetic and sympathetic nervous system, ATP is known to be co-released with ACh or noradrenaline (Richardson & Brown, 1987; Majid, Okajima & Kondo, 1992). Ninety-six per cent of DMV neurones sensitive to ATP also responded to ACh and medial habenula neurones also exhibit a response to both ATP and ACh (Edwards *et al.* 1992). In comparison with DMV, a smaller number of NTS neurones exhibited a sensitivity to both ATP and ACh. All locus coeruleus neurones tested responded to ATP using the slice patch technique (Shen & North, 1993) (Table 2). These results suggest that vagal preganglionic neurones represent a site where cholinergic and purinergic receptors are colocalized, and that locus coeruleus is another site where noradrenergic and purinergic receptors are colocalized. This indicates the uniform nature of the vagal preganglionic area and locus coeruleus with respect to the responses to ATP and ACh, and ATP and NA, respectively. In contrast, the NTS may contain a subpopulation of neurones that respond to both ATP and ACh, which could be accounted for by distinct groups of neurones participating in a variety of functions such as control of circulatory, respiratory and gastrointestinal mobilities, and taste, at the brainstem level. Although co-existence of ATP and ACh in the same terminal on the DMV neurone has not been identified, both ATP and ACh could be a functional co-transmitter on the DMV neurones.

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