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Purinergic control of the quail rectum: Modulation of adenosine 5′-triphosphate-mediated contraction with acetylcholine

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Abstract

Electrical field stimulation (EFS) induces frequency-dependent contractions of the longitudinal muscle of isolated quail rectum which were sensitive to tetrodotoxin. The aim of the present study was to investigate whether purinergic neurons are implicated in the response to nerve stimulation. The shape of the EFS-induced contractile response was different depending on stimulus frequency; low frequencies (0.5–2 Hz) induced fast monophasic contractions with a small subsequent relaxation; whereas higher frequencies (5–50 Hz) induced biphasic contractile response that comprised fast initial component (as in case of low frequency) and a slow delayed contractile component in addition to the relaxation that follows the fast contractile component. Prior application of atropine (10 μM) completely abolished the slow delayed component but significantly enhanced the fast initial contractile component. Physostigmine (1–10 μM) significantly enhanced the slow delayed component with an inhibitory effect on the initial fast component. The nonspecific purinergic receptor antagonist, suramin (100–500 μM) significantly inhibited the fast initial contractile component with no significant effect on the slow delayed one. Complete blockade of the fast component was achieved by prior application of a combination consisted of suramin (50 μM) and pyridoxicalphosphate-6-azophenyl 2′,4′-disulphonic acid tetrasodium (PPADS; 10 μM). Exogenous applications of adenosine 5′-triphosphate and acetylcholine (10 μM each), produced contractile responses that mimicked those induced by EFS. These data suggest that ATP is the main noncholinergic excitatory transmitter controlling the contractile activity of the quail rectum; and that its action could be modulated by acetylcholine.

Keywords: Quail; Rectum; Neuromuscular transmission; ATP; Acetylcholine

1. Introduction

Neurotransmitter(s) mediating the excitatory activity in fowl rectum is still a matter of debate since Takewaki and colleagues (Takewaki et al., 1977; Ohashi et al., 1977; Kanazawa et al., 1980) found that the rectum of fowl receives excitatory innervation from Remak's nerve, which is the most conspicuous nerve supplying fowl digestive tract. Although the neurotransmitter(s) has not been identified yet, neurotensin was proposed as a candidate based on the findings that neurotensin could be isolated from the rectum (Iwabuchi et al., 1987) and exogenously applied neurotensin produced mechanical and membrane responses similar to those to noncholinergic, nonadrenergic (NANC) nerve stimulation (Komori et al., 1986, 1992). Later, however, Unno et al. (1999) provided a contradictory evidence for the idea that neurotensin acts as a neurotransmitter of NANC nerves in the rectum of birds depending on the finding that SR48692, a non-peptide neurotensin receptor antagonist and thus antagonizes the contractile responses to exogenously applied neurotensin, was without effect on the nerve stimulation-evoked NANC contractile responses and excitatory junction potentials. Another trial was

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reported by Meldrum and Burnstock (1985) where they suggested that adenosine 5′-triphosphate (ATP) is the chemical transmitter to the chicken rectal smooth muscle. Their suggestion was dependent on the observation that prolonged exposure of the tissue to α,β-methylene ATP, a stable ATP analogue, which desensitizes the purinoceptors and thus renders smooth muscle insensitive to ATP, but not to carbachol, also abolished the contractile responses mediated by intramural nerve electrical stimulation.

Such previous trials have been done using chicken as experimental animal with no information on other fowl species including quail, which is considered now one of the most economic and productive species in poultry industry. The present study, therefore, was designed to shed some light on the excitatory neurotransmitter(s) controlling the quail rectum using tension recording technique and appropriate antagonistic drugs.

2. Materials and methods

2.1. Animals

Normal hybrid Japanese quails of either sex, aging 6–8 weeks of age were used in this study. There were no obvious differences in the results from animals of different sex. Normal hybrid Japanese quails were purchased from a commercial farm located in Gifu, Japan.

2.2. Rectum preparation

Quails were lightly anaesthetized with ether and killed by cervical dislocation. The abdominal cavity was opened and the whole rectum was removed together with Remak's nerve and caudal rectal vein. The content of the excised segment was gently flushed out using a small cannula containing Tyrode's solution (mM composition: NaCl 137; KCl 2.7; NaH_2PO_4 0.4; $NaHCO_3$ 12; $MgCl_2$ 1.0; $CaCl_2$ 1.8; glucose 5.0). One-cm-length of the rectum was cut 0.5 cm aboral to the cloaca to make a segment preparation (Fig. 1). The preparation was suspended vertically and longitudinally with the anal end uppermost in a 10 ml organ bath, filled with Tyrode's solution, which was aerated with atmospheric air and maintained at 35 ± 0.5 °C. The lower end of the preparation was fixed to the floor of the organ bath; the upper end was ligated by a thread, which attached to a transducer. The tissue was left to equilibrate for at least 45 min before starting the experiment.

2.3. Measurement of mechanical activity

Mechanical changes at the longitudinal direction of the preparations were recorded isometrically with a force displacement transducer (Orientec T7-30-240, Japan), AC amplifier (NEC-Sanei, AS1202, Japan), and a potentiometric pen recorder (Hitachi, 561, Japan). Electrical field stimulation (EFS) was carried out by means of two platinum-wire rings 0.4 mm in diameter which were connected to an electric stimulator (Nihon Koden, Sen-2201, Japan).

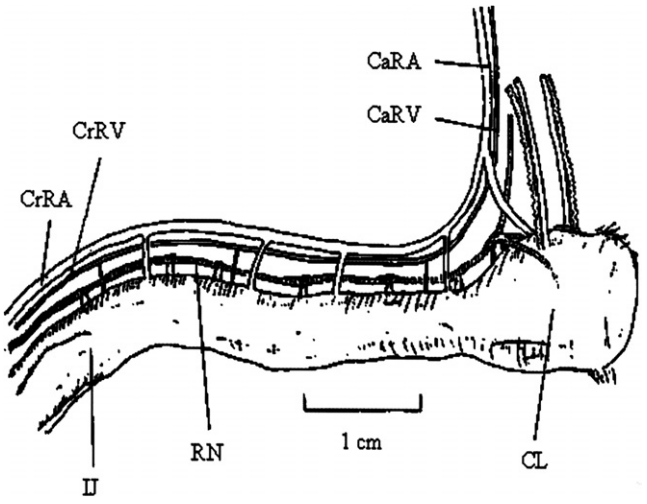

Fig. 1. Diagramatic sketch of the quail rectum showing the specimen used. RN: Remak's nerve; CaRA: Caudal rectal artery; CrRA: Cranial rectal artery; CaRV: Caudal rectal vein; CrRV: Cranial rectal vein; IJ: Ileocecocolic junction; CL: Cloaca.

2.4. Experimental protocol

EFS was used to deliver rectangular pulses at different frequencies. The pulse duration was fixed at 1.0 ms for stimulation of intramural nerves. The stimulus intensities were used at supra-maximal voltages at any particular frequency. Trains of pulses lasting for 5 s were delivered at intervals of not less than 5 min.

After equilibration, responses to EFS were recorded 10 times. The average amplitude of the responses to third and fourth stimulation and eighth and ninth stimulation were defined as S1 and S2, respectively. The preparations which have the unstable amplitude of the first five times stimulation were not used to the following experiments. Except when otherwise stated, drugs were added to the perfusing solution 15 min before the sixth stimulation. Reactive responses were expressed by comparing responses to S2 with those to S1.

2.5. Drugs

Drugs used and their sources were acetylcholine chloride, PPADS (pyridoxalphoshate-6-azophenyl 2′4′-disulphonic acid), tetrodotoxin (TTX), ATP (adenosine 5′-triphosphate), and physostigmine (Sigma chemicals, St Louis, USA), atropine sulfate monohydrate and suramin (Wako Pure Chemicals Industries, Osaka, Japan).

2.6. Statistical analysis

Data expressed as mean ± standard error of the mean, and "*n*" represents the number of different tissue preparations from different quails. The magnitudes of contractions

were expressed either as percentages of fast and slow contractions evoked by EFS at 10 Hz or as absolute values in grams (g). Differences between the means were analyzed by Student's *t*-test. $P < 0.05$ was considered significantly different.

3. Results

3.1. Responses to EFS

The isolated rectum exhibited spontaneous contractile activity or active muscle tone. EFS (1 ms duration for 5 s) of intramural nerves at low frequencies of 0.5–2 Hz produced fast, monophasic contractions. Whereas at higher frequencies of stimulation (5–50 Hz), two types of mechanical responses could be discriminated; a biphasic response in approximately 50% of rectal preparations ($n = 37$) and a triphasic one in the remaining preparations ($n = 34$). The biphasic response to EFS comprised an initial fast (simply it will be named fast) contraction and a subsequent relaxation. Whereas the triphasic responses to EFS consisted of the two components of the biphasic response followed by a delayed slow (simply it will be named slow) contraction (Fig. 2a). The excitatory and inhibitory responses were completely blocked after the application of TTX (1 μM), indicating that they are neurogenic in origin (data not shown; $n = 5$). The relationship between the stimulus frequency and the magnitude of contractile responses are shown in Fig. 2b. The fast contraction increased in magnitude as the stimulus frequency was increased, and the maximal responses were obtained at 10 Hz. The slow contraction was not observed at frequencies less than 5 Hz, but markedly achieved at 10 Hz.

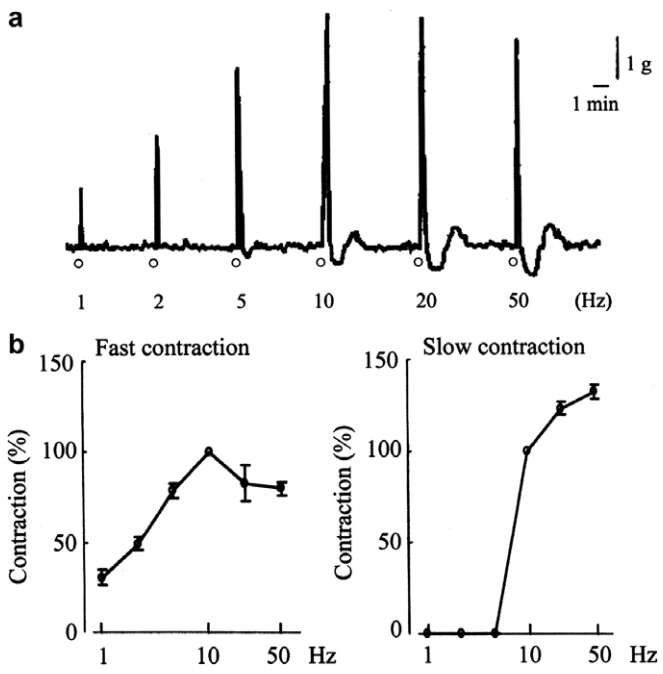

Fig. 2. Contractile responses to electrical field stimulation (EFS; ○) of the quail rectal strip. (a) Typical responses to EFS delivered at frequencies of 1–50 Hz. EFS at lower frequencies (1–2 Hz) evoked only a fast phasic contraction. However, EFS at higher frequencies (10–50 Hz) evoked the fast phasic contraction, a subsequent relaxation and a slow contraction. (b) Frequency–response curves of the fast and the slow contraction delivered at 1–50 Hz. The magnitudes of the fast and slow contractions were calculated as a percentage of the magnitudes of these contractions, respectively, that are evoked by EFS at 10 Hz. Each value is mean ± S.E.M; $n = 7$.

3.2. Effects of atropine and physostigmine on the contractile responses to EFS in quail rectum

Fig. 3a and b illustrates the biphasic and triphasic responses, respectively, of the rectal preparations evoked by a train of pulses at 10 Hz before and after application of atropine (10 μM). The fast contractions were appreciably larger in the presence of atropine ($n = 14$), but the slow contractions were completely blocked by the antimuscarinic agent ($n = 7$).

Physostigmine at concentrations of 1–10 μM slightly but significantly decreased the magnitude of the fast contractions ($n = 12$; Fig. 4). Preparations with biphasic responses started to exhibit the third component (slow contraction) after application of physostigmine ($n = 6$). Atropine (10 μM) overcame such potentiating effect produced by this cholinesterase inhibitor (data not shown; $n = 4$).

3.3. Effects of purinoceptor antagonists on NC contractile responses to EFS and exogenously applied ATP and ACh in quail rectum

The identity of the neurotransmitter mediating the fast contraction in responses to EFS of intramural nerves was then investigated. Suramin (100–500 μM), a non-selective P2 receptor antagonist, slightly inhibited the spontaneous activity of the rectal segment. The drug dose-dependently (data not shown) inhibited the fast contractions evoked by EFS. The significance of the blocking effect was evident at concentration of 100 μM ($n = 6$). Suramin was without effect on the slow contractions ($n = 5$).

A combination of suramin (50 μM) and PPADS (10 μM), another P2 antagonist, strongly inhibited or completely abolished the fast contraction ($n = 14$; Fig. 5), but had no effect on the cholinergic slow contraction ($n = 5$).

ATP (10 μM)-induced contractions were antagonized after the combined application of suramin (50 μM) and PPADS (10 μM); but, on the other hand, was enhanced by application of atropine (10 μM). ACh (0.1–10 μM)-induced contractile responses that were not significantly changed by the combination with the purinergic antagonists ($n = 4$) but blocked by atropine ($n = 4$; Fig. 6).

4. Discussion

The present findings suggest that NC contractile responses are the predominant excitatory responses in the rectal smooth muscle of the quail. The fast contractile responses to EFS appeared to be entirely mediated through

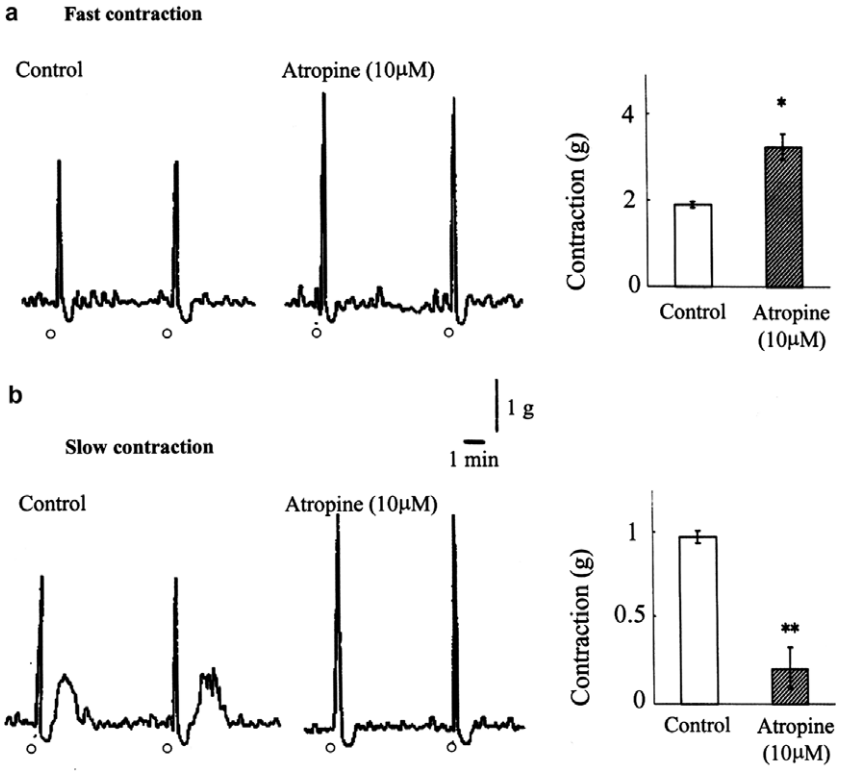

Fig. 3. Effect of atropine on EFS-evoked fast and slow contractions recorded from quail rectal strip. (a) Typical responses and histogram showing the biphasic response (fast contraction and subsequent relaxation) to EFS (○) at 10 Hz and the effect of muscarinic blocker atropine (10 μM). (b) Typical responses and histogram showing the triphasic response (fast contraction, relaxation and slow contraction) to EFS (○) at 20 Hz and the effect of the muscarinic receptor blocker atropine (10 μM). Note that atropine enhanced the amplitude of the fast contraction and markedly reduced that of the slow contraction. Each value is mean ± SEM; $n = 14$. $^{*}P < 0.05$; $^{**}P < 0.01$: significantly different from corresponding control (unpaired t-test).

excitation of NC nerves. The fast contractions evoked by stimulation of the extrinsic (Remak's) and the intramural nerves were unaffected by atropine, a muscarinic receptor blocker, indicating that they are not cholinergic in nature. It has been reported that the NANC contractions were evoked by stimulation to extrinsic nerve of the avian rectum and putative transmitters, such as histamine, serotonin, adenosine and prostaglandins, seem to be excluded as possible motor transmitters of the excitatory nerves (Bartlet, 1974; Takewaki et al., 1977).

The present results including the concurrent enhancing action of atropine to the NC fast contraction and the blocking action on the slow contraction may suggest the presence of prejunctional muscarinic receptors on the intramural nerves of Remak's nerves, and postjunctional muscarinic receptors on the smooth muscle cells of the quail rectum. Thus, the functional mechanisms of the NC excitatory neurons in the quail rectum may be regulated by cholinergic neurons. Stimulation of the cholinergic nerves with subsequent release of ACh may be responsible for the muscarinic inhibition of the NC contractile responses. The prejunctional inhibition by ACh via muscarinic receptors located on neurons has been reported previously for more than one set of neurons including cholinergic neurons itself (Kilbinger and Wagner, 1979) and adrenergic neurons as well (Kilinge and Sjostrand, 1977; Starke, 1981). In addition to its prejunctional modulatory role, ACh may be released upon application of electrical stimulation at high frequencies to intramural nerves of the quail rectum producing atropine- and physostigmine-sensitive contractile component that is mediated via its action on the muscarinic receptors located on the rectal smooth muscle cells (postjunctional effect).

The hypothesis of ATP as a NANC transmitter has been proposed after the observation that atropine-resistant responses to nerve stimulation in the urinary bladder, vas deferens and artery of mammals are mediated by ATP-release as the transmitter from the purinergic nerves (Burnstock, 1972). The present results suggest that ATP may act as the NC excitatory neurotransmitter in the quail rectum. The following observations are consistent with this view: (i) the combined application of suramin and PPADS; universal and P2X purinoceptor antagonists, respectively, completely inhibited the atropine-resistant fast induced contraction; (ii) ATP-induced contraction mimicked atropine-resistant contractile responses to nerve stimulation and that contraction was also blocked by the purinoceptor blockers; (iii) these antagonists did not alter the ACh-induced contraction. These findings indicate ATP to be considered as the neurotransmitter responsible for the fast contraction evoked by nerve stimulation of rectal smooth muscle of the quail.

Previously, Meldrum and Burnstock (1985) reported that ATP and the non-degradable ATP analogue, α,β-

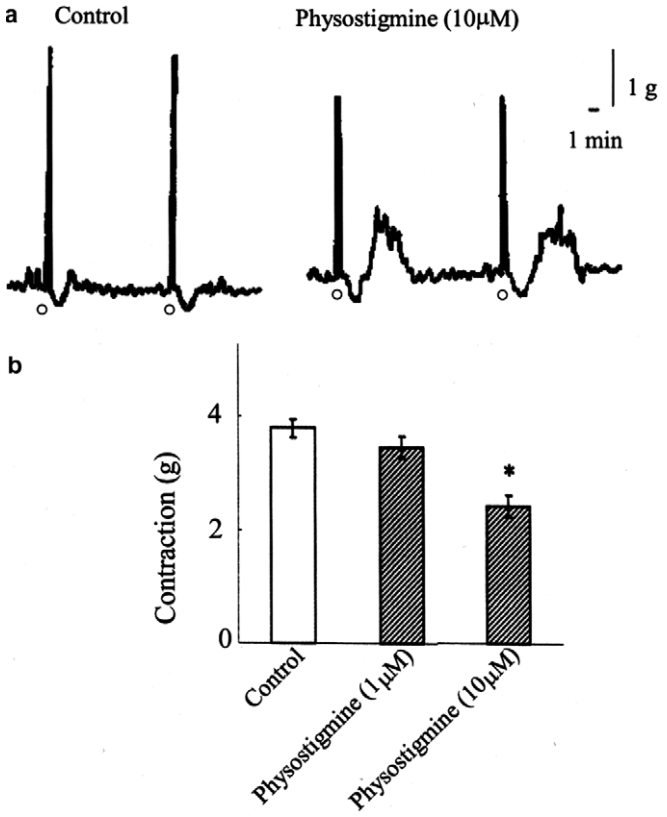

Fig. 4. Effect of physostigmine on EFS-evoked fast and slow contractions recorded from quail rectal strip. (a) Typical responses showing the triphasic response (fast contraction, relaxation & slow contraction) to EFS (○) at 10 Hz (left panel) and the effect of cholinestrase enzyme inhibitor physostigmine (10 μM) (right panel). (b) Histogram showing the dose dependent effect of physostigmine (1–10 μM) on the fast contraction evoked by EFS. Note that physostigmine enhanced the amplitude of the slow contraction but inhibited the slow one. $n = 12$. $^{*}P < 0.05$: significantly different from corresponding control (unpaired t-test).

methylene ATP, mimicked in their action the response of the chicken rectum to nerve stimulation. After desensitization of the P2-purinoceptors by α,β-methylene ATP, ATP- and nerve stimulation- induced contractions were not evident. Therefore, they concluded that ATP may be the transmitter in NANC excitatory nerves supplying the chicken rectum. However, Komori et al. (1988) also reported that the suppression by α,β-methylene ATP of the chicken rectum is due to a change in the electrical properties of the membrane of the smooth muscle cells, rather than due to desensitization of the purinoceptor. Since then, the NANC excitatory transmitter to the chicken rectum is still a controversial issue. In this report we clarified that the neurotransmitter in a related species (quail) is ATP depending on the data of the well established purinoceptor antagonists suramin and PPADS. ATP is a ligand for P2 purinoceptors existing in two main subtypes: (1) the P2X receptors that are ligand-gated ion channels and (2) the P2Y receptors that are coupled to G proteins (Ralevic and Burnstock, 1998). Excitatory responses are assumed to be mediated mainly through P2X receptors, while inhibitory responses are assumed to be mediated mainly through P2Y ones. Every receptor group includes many subtypes based on their molecular structure, location and transduction pathways. Determination of the receptor subtype involved in the fast contractile response in the quail rectum remains to be investigated in a next report.

Earlier in Section 4, we have discussed that ACh may act as a regulator to another motor neuron(s). Now and after the evidence provided in this study that this other motor neuron is purinergic in nature and ATP is the main NC transmitter to the quail rectum, it could be stated that a muscarinic receptor population might be present on the purinergic nerve prejunctional terminals which when stimulated with ACh, it decreases ATP release and thus inhibits fast contractions. In addition, a postjunctional modulatory mechanism may also occur along the course of P2 receptor involved in the fast contraction indicated by the enhancing effect of atropine on the contractile response evoked by exogenously applied ATP.

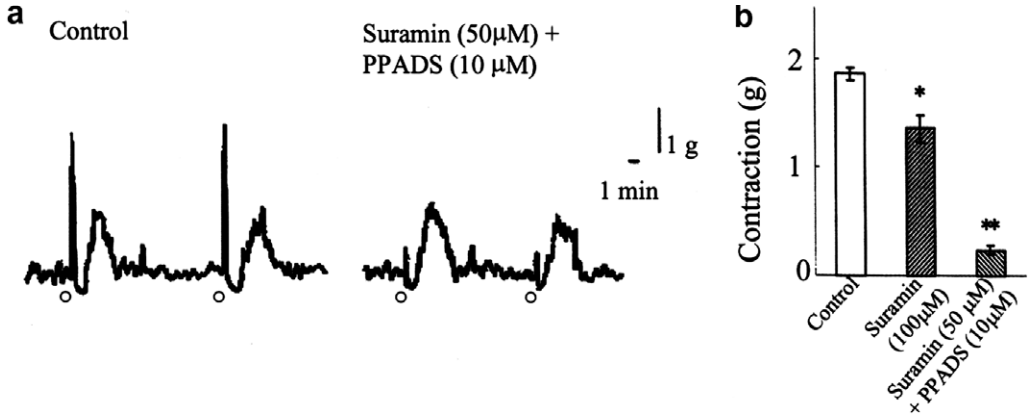

Fig. 5. Effect of purinergic antagonists (suramin and PPADS) on EFS-evoked fast and slow contractions recorded from quail rectal strip. (a) Typical responses showing the triphasic response (fast contraction, relaxation and slow contraction) to EFS (○) at 10 Hz and the blocking effect of suramin (50 μM) plus pyridoxicalphosphate-6-azophenyl 2′,4′-disulphonic acid tetrasodium (PPADS; 10 μM). (b) Histogram summarising the effect of suramin (100 μM) and combination of suramin (50 μM) plus PPADS (10 μM) on EFS-mediated fast contraction. Note that purinergic antagonists markedly inhibited the amplitude of the fast contractions. Each value is mean ± SEM; $n = 6$. $^{*}P < 0.05$; $^{**}P < 0.01$: significantly different from corresponding control (unpaired t-test).

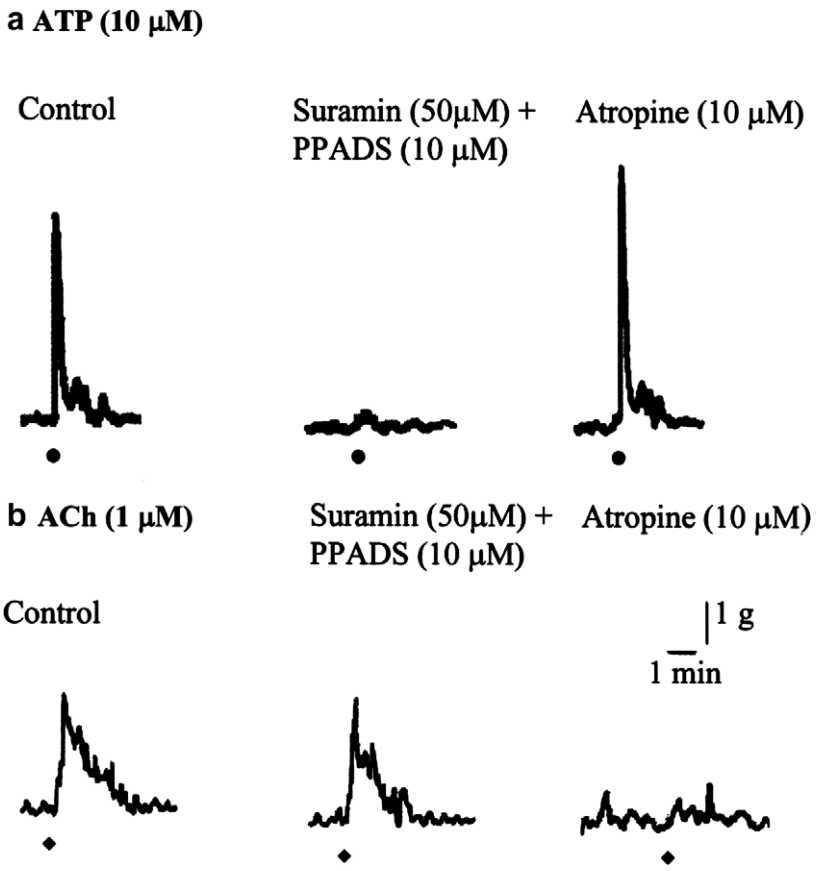

Fig. 6. Contractile effects of exogenously applied ATP (10 μM) and ACh (1 μM) on quail rectal strip and the effects of purinergic and cholinergic antagonists. (a) Typical tracings showing the contractile response after 10 μM ATP mimicking the fast contraction evoked by EFS; the blocking effect of suramin (50 μM) plus PPADS (10 mM); and the enhancing effect of atropine (10 μM). ● denotes application of ATP. (b) Typical tracings showing the contractile response after 1 μM ACh mimicking the slow contraction evoked by EFS; the non-effect of suramin (50 μM) plus PPADS (10 μM); and the blocking effect of atropine (10 μM). ♦ denotes application of ACh.

In conclusion, our results give evidence that ATP may be the main NC excitatory transmitter to the quail rectal smooth muscle. Contributing and modulatory roles for ACh were also suggested.

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