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Antagonism of P2X receptors in guinea-pig vas deferens by diinosine pentaphosphate

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Abstract

Diinosine pentaphosphate (Ip_5I) antagonized contractions, mediated via P2X receptors, evoked by diadenosine pentaphosphate (Ap_5A) and ATP in the guinea-pig isolated vas deferens with pA_2 values of 6.4 ± 0.17 (10 d.f.) and 6.5 ± 0.10 (10 d.f.), respectively. Ip_5I (30 μM) did not affect contractile responses evoked by noradrenaline. Ip_5I (up to 100 μM) did not antagonize P2Y receptors in the guinea-pig taenia coli, nor P1 or P2 receptors in the guinea-pig left atrium. © 1997 Elsevier Science B.V.

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P^1,P^5-bis(5′-inosyl)pentaphosphate (diinosine pentaphosphate, Ip_5I) is closely related to the P^1,P^n-bis(5′-adenosyl)oligophosphates (diadenosine polyphosphates), and was synthesised by deaminating the adenosine moieties of diadenosine pentaphosphate (Ap_5A) (Guranowski et al., 1995; Pintor et al., 1997). The identity and purity of Ip_5I were confirmed and assessed by high performance liquid chromatography (Pintor et al., 1997). Ip_5I has been shown to antagonize the dinucleotide receptor, which is specific for diadenosine polyphosphates, in synaptosomal preparations from the rat brain with an IC_{50} around 4 nM, and antagonises the P2 receptor in the same preparation with an IC_{50} around 30 μM (Pintor et al., 1997).

Mid-segments of vasa deferentia (1.5 cm long) from guinea-pigs (420–485 g) were suspended vertically in 5 ml organ-baths, in Krebs solution (37°C), as were segments of taenia coli (1.5 cm long). The left atrium was prepared and paced as described previously (Hoyle et al., 1996). Mechanical activity was recorded isometrically.

In the vas deferens, at concentrations up to 100 μM, Ip_5I had no contractile effect, while Ap_5A (0.1–30 μM) and ATP (1.0–1000 μM) evoked concentration-dependent contractions that are mediated via P2X receptors (MacKenzie et al., 1988; Hoyle et al., 1995). In the presence of Ip_5I (0.1–10 μM) the concentration–response curves for the two agonists became displaced towards the right (Fig. 1a). Noradrenaline (0.3–100 μM) also evoked concentration-dependent contractions, but these were not significantly affected by Ip_5I at 30 μM. The Schild plot for Ap_5A (Fig. 1b) was significantly linear, with a slope of 0.84 ± 0.17 (not significantly different from unity, 10 d.f.), yielding a pA_2 value for Ip_5I of 6.4 ± 0.17 (10 d.f.). The corresponding plot for ATP was not significantly different from that for Ap_5A (two-way ANOVA) yielding a pA_2 value for Ip_5I of 6.5 ± 0.10 (10 d.f.). Thus Ip_5I appears to be a surmountable antagonist with a submicromolar IC_{50}. These pA_2 values compare with pIC_{50} values of 8.4 and 4.6 against Ap_5A and ATP in rat brain synaptosomes (Pintor et al., 1997). Whereas this rat brain preparation has two distinct receptors, one for diadenosine polyphosphates and one for ATP and related mononucleotides (Pintor and Miras-Portugal, 1994, 1995), the guinea-pig vas deferens does not appear to do so.

In the guinea-pig taenia coli, contracted by carbachol (50 nM) to achieve a standard level of tone, Ap_5A and ATP at concentrations that caused approximately 50% relaxation (mediated via P2Y receptors), were not significantly affected by Ip_5I (30 μM) ($P < 0.05$, paired *t*-tests before and after 30 min incubation, $n = 4$). In the guinea-pig driven left atrium, where Ap_5A and ATP cause a negative inotropy mediated via P1 receptors (Hoyle et al.,

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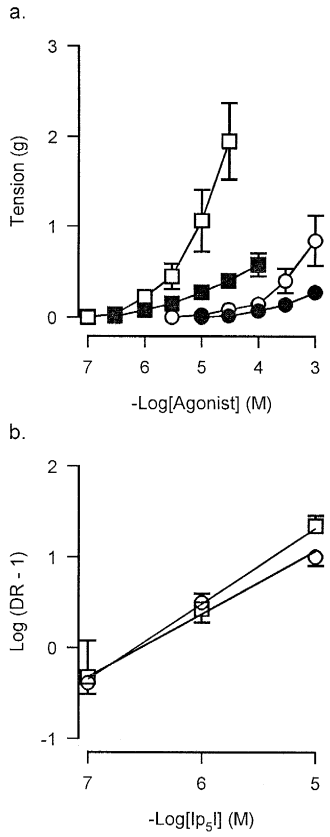

Fig. 1. Antagonism of diadenosine pentaphosphate (Ap_5A) and ATP by diinosine pentaphosphate (Ip_5I) in the guinea-pig isolated vas deferens. (a) Concentration–response relationships for Ap_5A (open squares) and ATP (open circles) in the absence of Ip_5I, and in the presence of Ip_5I (10 μM, corresponding closed symbols). (b) Schild plots Ip_5I against Ap_5A (open squares) and ATP (open circles). Lines are calculated linear regressions. Points show mean $\pm$ S.E., unless occluded by symbol. All points $n = 4$.

1996), Ip_5I up to 100 μM also had no effect ($P < 0.05$, paired *t*-tests before and after 30 min incubation, $n = 4$). Also in the left atrium, in the presence of 8-*p*-sulphophenyltheophylline (100 μM), Ap_5A (100 μM) evokes positive inotropic responses, via an ill-defined P2 receptor (Hoyle et al., 1996). These were also unaffected by Ip_5I (100 μM) ($P < 0.05$, paired *t*-tests before and after 30 min incubation, $n = 4$).

In conclusion, Ip_5I is a selective antagonist of the P2X receptor in the guinea-pig vas deferens without apparent antagonistic activity at either P1 or P2Y receptors. It is suggested that Ip_5I represents a new class of P_2-purinoceptor antagonist, and that it may prove to be a valuable starting point in the development of novel antagonists.

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