Diinosine Polyphosphates, a Group of Dinucleotides with Antagonistic Effects on Diadenosine Polyphosphate Receptor

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SUMMARY
A new family of dinucleotide derivatives, diinosine polyphosphates, has been synthesized through the use of the enzyme 5′-adenylic acid deaminase from Aspergillus sp., starting from the corresponding diadenosine polyphosphates. Functional studies were performed on rat brain synaptic terminals in which a dinucleotide receptor has been described that is specific for adenine dinucleotides. The results demonstrated that diinosine polyphosphates did not behave as agonists on the diadenosine polyphosphate receptor (also known as P₄ purinoceptor), but they were very efficient as antagonists in abolishing the Ca²⁺ responses elicited by diadenosine pentaphosphate. The IC₅₀ values for diinosine triphosphate, diinosine tetraphosphate, and diinosine pentaphosphate were 4.90 ± 0.10 μM, 8.33 ± 0.22 μM, and 4.23 ± 0.12 μM, respectively. The diinosine polyphosphates also antagonized the ATP receptors present in synaptic terminals, showing IC₅₀ values of 100.08 ± 5.72 μM for diinosine triphosphate, 29.51 ± 1.40 μM for diinosine tetraphosphate and 27.75 ± 1.65 μM for diinosine pentaphosphate. The antagonistic ability of these diinosine nucleotides was studied in comparison with other P₄ and P₂ purinoceptor antagonists, such as suramin, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid and 8-cyclopentyl-1,3-dipropylxanthine. These purinergic antagonists did not inhibit the response of the P₄ purinoceptor; only the diinosine polyphosphates were able to act as antagonists on the dinucleotide receptor. Suramin and pyridoxal phosphates-6-azophenyl-2′,4′-disulfonic acid attenuated the responses elicited by ATP, as did the diinosine polyphosphate compounds. The most antagonistic diinosine polyphosphate for the dinucleotide and ATP receptors was diinosine pentaphosphate, which was 6000 times more selective for the P₄ purinoceptor than it was for the ATP receptor.

ApₙAs (where n ranges from three to six phosphates) are a family of compounds formed by two adenosine moieties bridged by a variable number of phosphates. These compounds are active substances in neural and non-neural tissues (1). Their action is generally mediated by ATP purinergic receptors and in some cases by adenosine-like receptors in specific areas of the central nervous system (2, 3). Recently, a new subtype of presynaptic receptor that is exclusive to ApₙAs has been described in the central nervous system (4). This receptor, characterized in rat brain synaptic terminals, has tentatively been termed P₄ purinoceptor, or dinucleotide receptor. The activation of the P₄ purinoceptor by Ap₄A and Ap₆A is coupled to the Ca²⁺ entry to the synaptic terminals via a voltage-independent mechanism. The main feature of the dinucleotide receptor is the nonsensitivity to ATP and its synthetic analogs, being activated only by Ap₄As (5). Very recently, a receptor sensitive to Ap₄A was also described in deer mouse brain synaptosomes (6).

Receptors for ApₙAs and adenine nucleotides have been pharmacologically characterized by means of ATP synthetic analogs due to the scarcity and poor specificity of the available antagonists (7). The use of agonistic profiles has not allowed a clear characterization of the receptors present in different tissues. Several compounds have been tested as P₂ antagonists, among which suramin and PPADS are still the most widely used (8, 9). Although initially both were used to discriminate within different P₂ purinoceptor subtypes, only PPADS seems to be quite specific for the P₂X purinoceptors, with suramin suitable for distinguishing between P₁ and P₂ purinoceptors (10).

The existence of nonspecific adenylate deaminases isolated from organisms such as the snail Helix pomatia and microorganisms such as Aspergillus sp. allows the enzymatic transformation of the ApₙAs and adenosine nucleotides to inosine.

ABBREVIATIONS: Ap₄A, diadenosine polyphosphate or adenine dinucleotide; Ip₃I, diinosine triphosphate; Ip₄I, diinosine tetraphosphate; Ip₅I, diinosine pentaphosphate; Ap₉As, diadenosine polyphosphate; IpₙI, diinosine polyphosphate; PPADS, pyridoxal phosphates-6-azophenyl-2′,4′-disulfonic acid; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; HPLC, high performance liquid chromatography; Ap₄A, diadenosine pentaphosphate; Ap₆A, diadenosine tetraphosphate; Ip₃I, diinosine triphosphate; Ip₄I, diinosine tetraphosphate; Ip₅I, diinosine pentaphosphate; HEPES, 4-(2-hydroxyethyl)1-piperazineethanesulfonic acid; TES, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.
derivatives (11). The transformation of ApnA by adenylate deaminases yields compounds with the structure inosine(5’)-oligophosphate(5’)-inosine (Ip, I). The Ip, I’s could enlarge the number of different nucleotidic compounds acting as agonists or antagonists on purinergic receptors, especially on the P2 purinoceptor, due to their structural similarities to ApnA.

In the current study, we describe the synthesis of Ip, I from Aspergillus sp. by means of a commercial AMP deaminase. The relevant antagonistic properties of these dinucleotides on the P2 purinoceptor and ATP receptors is reported in rat brain synaptic terminals. Special attention is devoted to Ip, I because its high antagonistic activity on ApnA receptors makes this compound a useful tool with which to discriminate between ATP receptors and P4 purinoceptors.

Experimental Procedures

Synthesis of Ip, I’s. Ip, I’s were obtained by starting with the corresponding ApnAs through the action of 5’-adenylic acid deaminase from Aspergillus sp. The enzyme (0.12 unit) was incubated with 10 mM ApnA, ApnA, or ApnA in a final volume of 1 ml of 50 mM HEPES, pH 6.5. Incubation was carried out at 37°. Aliquots of 10 µl were taken at different times, placed in a 100° water bath for 5 min (to stop the reaction), and diluted 1/100 with distilled water before injection into the HPLC system to follow the process of deamination. After 1.5 h, the reaction was finally stopped by boiling the incubation mixture at 100° for 5 min followed by centrifugation at 13,000 × g to eliminate the protein debris. Boiling did not affect the stability of the formed Ip, I as previously described for ApnAs (12).

Treatment with phosphodiesterase from Crotilus durissus (EC 3.1.15.1) was carried out to verify the nature of the formed compounds. Aliquots of 15 µl of the final reaction mixture were diluted 1:100 with distilled water and incubated with 3 munits of phosphodiesterase at 37°. Samples of 20 µl were taken, at different times, to follow the appearance of the inosine mononucleotides by HPLC.

Chromatographic procedures. The chromatographic equipment consisted of a Waters (Milford, MA) 600E system controller, a Waters 717+ autosampler, and a 481 Amax spectrophotometer, all of which were managed by Millenium 2010 software running on an NEC 486DX computer. Analysis was performed under ion-pair chromatography conditions by equilibrating the chromatographic system with the following mobile phase: 0.1 mM KH2PO4, 2 mM tetrabutylammonium hydrogen sulfate, and 17% acetonitrile, pH 7.4. The cytosolic free calcium concentration was determined using Fura-2 as described by Grynkiewicz et al. (13). Five minutes after resuspension, 1.33 mM CaCl2 and 5 mM Fura-2-acetoxymethyl ester were added. After incubation for 35 min, the synaptosomes were pelleted (through centrifugation at 800 rpm for 1 min), washed twice, and resuspended in fresh medium containing 1.33 mM CaCl2. Fluorescence was measured in a Perkin-Elmer Cetus (Norwalk, CT) Spectrofluorimeter LS-50 and monitored at 340 and 510 nm. Data were collected at 0.5-sec intervals.

Pharmacological studies. ApnA and ATP were tested at a final concentration of 100 µM. Also, Ip, I’s were assayed at the same concentration before any antagonistic experiment was performed. The capacities of Ip, I, Ip, I, and Ip, I to act as possible antagonists was analyzed by assaying them at different concentrations ranging from 10−10 to 10−3 M. When tested as antagonists, Ip, I’s were preincubated 2 min before the addition of the agonists. Other P2 antagonists, such as suramin and PPADS, were assayed at a final concentration of 100 µM 2 min before the application of the agonist. DPCPX, an A1 adenosine antagonist, was preincubated at a final concentration of 250 nM at 2 min before the agonist was added to the synaptosomal preparation.

Studies were also performed to obtain the equilibrium dissociation constant (Kd) for Ip, I. A series of ApnA dose curves (ranging from 10−10 to 10−8 M) were obtained in the presence of Ip, I at the following concentrations: 0, 4, 40, and 400 nM. These curves were analyzed according to the method of Stone and Angus (14) to obtain the Kd value for the antagonist.

Materials. ApnA, ApA, ApA, ATP, ITP, IDP, IMP, and 5’-adenylic acid deaminase from Aspergillus sp. were obtained from Sigma Chemical (St. Louis, MO). Suramin was kindly provided by Dr. A. Ijzerman (Center for Drug Research, Division of Medicinal Chemistry, Leiden, The Netherlands). PPADS and DPCPX were purchased from Research Biochemicals (Natick, MA). Phosphodiesterase from C. durissus (EC 3.1.15.1) was purchased from Boehringer-Mannheim Biochemica (Mannheim, Germany). Fura-2 was obtained from Molecular Probes (Eugene, OR). Other analytical-grade reagents were purchased from Merck (Darmstadt, Germany).

Statistical analysis. Data are presented as mean ± standard error (for IC50 values) of curves fitted by means of Fig P version 6.0 (Biosoft, Cambridge, UK). Experiments were performed at least four times in duplicate and in different synaptosomal preparations. Significant differences were determined by two-tailed Student’s t test. When appropriate, single experiment traces are represented in the figures; they are representative of at least four determinations in duplicate with equivalent results.

Results

Chromatographic characterization of Ip, I’s. Ip, I’s were synthesized by starting with ApnAs through sequential deamination of both adenosine moieties. The 5’-adenylic acid deaminase from Aspergillus sp. was very efficient with the three ApnAs that were converted to the corresponding Ip, I (Fig. 1). The reaction had a yield of 100% after 1.5 hr with no further transformation of the products into other compounds. At the intermediate times, it was possible to note the presence of peaks with retention times between those of the initial ApnA and the final Ip, I. It seems possible that those peaks could correspond to the intermediate reaction products in which one of the adenosines remain unchanged while the other had already been deaminated (Fig. 1).

To verify the nature of the final products, samples of the putative Ip, I’s were collected and incubated with phosphodiesterase from C. durissus. This enzyme cleaves dinucleotides giving a nucleotide monophosphate plus another mononucleotide with an n−1 phosphate. The presence of IMP and IDP was determined for the putative Ip, I (Fig. 2A), of IMP and ITP for Ip, I (Fig. 2B), and of IMP and a compound putatively identified as inosine-5’-tetraphosphate for Ip, I (Fig. 2C). In all of the Ip, I compounds treated with the phosphodiesterase, a gradual increase in the inosine mononucleotides and a disappearance of the dinucleotide were observed. Once the identity of the synthesized Ip, I was confirmed, they were assayed as active molecules in rat brain synaptic terminals.

Effect of Ip, I’s on rat brain synaptic terminals. The application of Ip, I’s to the rat brain synaptic terminals in a concentration of ~100 µM did not induce any Ca2+ movement.
Fig. 1. Enzymatic synthesis of inosine(5')oligophospho(5') inosines occurred with reverse-phase chromatography. A, HPLC elution profile for the synthesis of Ip$_3$I. Ap$_3$A (10 mM) was incubated with Aspergillus sp. adenylylate deaminase. Aliquots of the reaction mixture were taken at different incubation times (0, 15, 45, and 90 min). Then, the reaction was stopped, and HPLC was used to follow the reaction development, as described in Experimental Procedures. The HPLC profiles obtained at increasing incubation times are represented as consecutive chromatograms, starting from time zero (rear trace). At low times, hybrid compounds are formed as a consequence of the partial deamination of the Ap$_3$A. Ip$_3$I was the only compound present after a 90-min incubation (front trace). B, Chromatographic profile of the Ip$_4$I synthesis. Ap$_4$A (rear trace) transformation was followed after 15, 45, and 90 min (front trace) of incubation with Aspergillus sp. deaminase, as described in A for the Ip$_3$I formation. C, Analysis of the Ip$_5$I formation under the same conditions as described in A and B.

Fig. 2. Characterization of Ip$_n$I through phosphodiesterase digestion. Putative Ip$_n$I were incubated with phosphodiesterase from C. durissus, as described in Experimental Procedures. Aliquots taken at 0, 3, 6, 10, and 20 min were injected and analyzed by ion-pair chromatography to follow the Ip$_n$I disappearance and the appearance of the corresponding degradation products. A, Characterization of Ip$_3$I through digestion with phosphodiesterase. Putative Ip$_3$I was treated as previously described. The consecutive chromatograms correspond to the HPLC profiles obtained at increasing digestion times. The disappearance of the Ip$_3$I is concomitant with the appearance of peaks of their degradation products (IMP and IDP). B, Characterization of Ip$_4$I through digestion with phosphodiesterase. Putative Ip$_4$I was analyzed as described in A for Ip$_3$I. The consecutive chromatograms show the formation of ITP and IMP from Ip$_4$I through incubation at increasing times with phosphodiesterase. C, Characterization of Ip$_5$I through digestion with phosphodiesterase. Putative Ip$_5$I was treated in the manner described for Ip$_4$I and Ip$_3$I. Digestion of Ip$_5$I peak with phosphodiesterase yields IMP and another metabolite with a retention time higher than that for ITP. This compound has been putatively identified as inosine-5'-tetraphosphate.
as a consequence of receptor activation (Fig. 3A). Ap5A assayed at a concentration of 100 μM elicited a Ca\(^{2+}\) increase of 30.51 ± 3.56 nM, as shown in Fig. 3A (basal synaptosomal Ca\(^{2+}\) concentration before the agonist application, 115.08 ± 5.12 nM).

Because the Ip\(_n\)Is did not exert any agonistic effect on the rat brain synaptic terminals (Fig. 3A), their capacity to act as a possible antagonists was tested. The incubation of the three Ip\(_n\)Is at a final concentration of 100 μM at 2 min before the Ap5A application reduced the Ca\(^{2+}\) signal elicited by the Ap5A (Fig. 3B). The treatment of synaptic terminals with other purinergic antagonists is shown in Fig. 3B. Suramin and PPADS, also at final concentrations of 100 μM, did not mimic the effect of Ip\(_n\)I. Suramin did not significantly modify the Ca\(^{2+}\) entry induced through the P\(_4\) purinoceptors, and PPADS surprisingly induced an enhancement of the signal compared with control (Fig. 3B). Similar behavior to that exhibited by PPADS was shown by DPCPX (250 nM), which is known to antagonize adenosine receptors.

**Antagonistic effect of Ip\(_n\)Is on the P\(_4\) purinoceptor.** Because Ip\(_n\)Is behaved as antagonists on the dinucleotide receptor, they were preincubated in a wide range of concentrations in the presence of 100 μM Ap5A. The Ca\(^{2+}\) responses elicited by Ap5A were attenuated by all Ip\(_n\)Is to various degrees depending on the concentration and the dinucleoside compound assayed. An example of the antagonistic effect of one of these dinucleotides, Ip5I, is shown in Fig. 4A, in which a gradual attenuation of the Ca\(^{2+}\) signal by higher doses of the Ip5I can be observed. Following a similar protocol inhibition, curves were obtained and analyzed to determined IC\(_{50}\) val-

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**Fig. 3.** Antagonistic effect of Ip\(_n\)Is and purinergic antagonists on P\(_4\) purinoceptors present in rat brain synaptic terminals. A, Effects of Ip3I, Ip4I, and Ip5I on calcium entry into rat brain synaptosomes evoked by 100 μM Ap5A. Effects of Ip3I and Ap5A on Ca\(^{2+}\) responses induced through the dinucleotide receptor (top two traces). The effect of Ap5A was blocked by the three Ip\(_n\)Is applied at a final concentration of 100 μM (bottom three traces). B, Comparative behavior of Ip\(_n\)Is and purinergic antagonists. All compounds were assayed at a final concentration of 100 μM, except DPCPX, which was applied at a concentration of 250 nM, modifying the Ap5A response in a different way. All experiments represent the mean ± standard error of four different experiments performed in duplicate. ***, p < 0.01 versus control; ***, p < 0.001 versus control.

**Fig. 4.** Effects of Ip\(_n\)Is on the responses elicited by 100 μM Ap5A. A, Increasing doses of Ip5I produced a gradual blocking of the responses elicited by Ap5A. A 30 mM K\(^+\) pulse is given at the end of each record as a control of the synaptosomal functional response. B, Effect of Ip3I, Ip4I, and Ip5I on the Ca\(^{2+}\) transients elicited by 100 μM Ap5A. Graded concentrations of the Ip\(_n\)I were added to obtain the corresponding responses. Ordinates, Ca\(^{2+}\) transients as a percentage of the control value for 100 μM Ap5A in the absence of any substance. Values are mean ± standard error from four experiments performed in duplicate.
ues for the three compounds (Table 1). It was noteworthy that Ip₃I was the most potent antagonist for the Ap₅A effect, with an IC₅₀ value in the nanomolar range (Table 1). Ip₄I and Ip₅I had IC₅₀ values in the micromolar range (Fig. 4B).

Various Ap₅A series of dose-response curves were assayed in the presence of different concentrations of Ip₅I to obtain the inhibition constant (Kᵦ) of the antagonist for the receptor. The concentration-response analyses for Ap₅A were performed in both the absence and the presence of the following concentrations: 4, 40, and 400 nM Ip₅I (equivalent to IC₅₀, 10-fold IC₅₀, and 100-fold IC₅₀ value, respectively). As displayed in Fig. 5A, there was a gradual displacement of the dose-response curves to the right that was directly related to the increase in the antagonist concentration. EC₅₀ values were analyzed according to the Stone and Angus equation and represented as a Clark plot (Fig. 5, B and C). The results obtained after the analysis yielded a Kᵦ value for Ip₅I of 78.9 ± 9.9 nM and a slope of 1.0 ± 0.1, indicating the existence of a competitive antagonist mechanism.

**Effects of IpₙIs on ATP responses.** The antagonistic properties of the IpₙIs were also assayed for the ATP responses in isolated rat brain synaptic terminals. ATP (100 μM) elicited a Ca²⁺ increase of 25 ± 2.7 nM (basal synaptosomal Ca²⁺ concentration before the agonist application, 115.08 ± 5.12 nM).

Ip₃I, Ip₄I, and Ip₅I antagonized the responses elicited by ATP, with IC₅₀ values in the micromolar range (Fig. 6 and Table 1). Ip₅I antagonized the Ca²⁺ transients elicited by ATP, but the concentration required to attenuate the response compared with Ap₅A was 6000 times higher, indicating that this Ip₅I behaves as a good tool with which to discriminate between P₄ and P₂ receptors in rat brain synaptic terminals (Fig. 6A).

**Inosine mononucleotide effect on Ap₅As and ATP Ca²⁺ transients.** To find out whether inosine mononucleotides could mimic the effect of IpₙIs on both the dinucleotide and ATP receptor, ITP, IDP, and IMP were assayed as agonists/antagonists on the Ca²⁺ transients elicited by 100 μM Ap₅A and ATP. ITP induced a Ca²⁺ transient that did not abolish a secondary Ca²⁺ response triggered by Ap₅A. This result suggested that both nucleotides activate different receptors and that the antagonistic effect of the IpₙI compounds is indeed caused by them rather than by their degradation products (Fig. 7A). None of the other inosine mononucleotides affected the responses elicited by Ap₅A (Fig. 7B). The application of ITP to the synaptosomal preparation before ATP produced a Ca²⁺ increase that blocked the responses induced by ATP, suggesting that ITP and ATP share a purinergic receptor (Fig. 7A). The other inosine mononucleo-

### TABLE 1

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<th>Ap₅A response</th>
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<tr>
<td>Ip₃I</td>
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<td>Ip₄I</td>
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Fig. 5. Effect of different Ip₅I concentrations on Ap₅A dose-response curve. A, Effect of Ip₅I (○, 0 nM; ■, 4 nM; ▲, 40 nM, and ▲, 400 nM) on Ap₅A Ca²⁺ transients. Data are fitted to logistic curves. B, Effect of increasing concentrations of Ip₅I on Ap₅A pEC₅₀ value. Agonist potency data were analyzed using nonlinear regression to yield the pKᵦ estimate. C, Clark plot displaying the effect of Ip₅I on Ap₅A pEC₅₀ value.
Discussion

The results that we present describe a simple method to synthesize Ip₃I by starting with Ap₅A. The synthesis procedure is similar to that described by Guranowski et al. (11), but a commercial 5’-adenylic acid deaminase from Aspergillus sp. is used instead of one from the snail Helix pomatia. The effects of Ip₃I, Ip₄I, and Ip₅I were tested on rat brain synaptic terminals, either alone or in the presence of Ap₅A and ATP.

The lack of an action on the Ca²⁺ transients by the three compounds suggested to us that they be studied as antagonists on the purinoceptors already characterized in rat brain synaptosomes (5). The three IpₙIs completely blocked the effect of the best P₄ purinoceptor agonist (Ap₅A). Ip₃I and Ip₄I presented a quite similar IC₅₀ value, in the micromolar range, that was higher than that exhibited by Ip₅I, which was in the nanomolar range. This fact indicates some degree of structural requirement for the dinucleotide receptor, which is fulfilled by Ip₅I (the closer structural antagonist compared with the best agonist, Ap₅A) but not so well by the other antagonists. Extremely important is the IC₅₀ value for Ip₅I, which was ~3 orders of magnitude lower than the micromolar values presented by the other diinosine compounds. Actions of Ap₅A in the nanomolar range have been previously reported in neural models. For example, the neu-
ral P2×x purinoceptor expressed in *Xenopus laevis* oocytes is positively modulated by Ap5A. This dinucleotide allosterically potentiates the ATP response with an EC50 value of 2.95 nM (15). Autoradiographic studies carried out with [3H]Ap4A at a concentration of 1 nM demonstrated specific binding of sites on rat enkephalin for this dinucleotide (16). These results correspond very closely to those obtained previously during experiments performed in rat brain synaptosomes in which Ap6As had *Kd* values in the low nanomolar range (17).

Suramin and PPADS did not follow the antagonistic behavior displayed by the Ip1.1 on the P4 purinoceptor. These results clearly contrast with those described in guinea pig urinary bladder, in which the effect of Ap4A is inhibited by suramin and especially by PPADS (18). In this model and in the vas deferens, Ap6As seem to activate a P2×x purinoceptor (19, 20), explaining why both suramin and PPADS antagonized the effect of Ap4A.

The effect of Ap4A was not blocked by the adenosine receptor antagonist DPCPX. This is an interesting point because in some central locations, such as the cerebral cortex and the hippocampus, the effect of Ap4A can be blocked by methylxanthines (2, 3). Nevertheless, a more detailed study on single CA3 hippocampal neurons revealed that extracellular Ap5A induced a Ca2+ inward current that was not blocked by A1- and A2-specific antagonists. CA3 neurons possess dinucleotide receptors that have the same features as the purinoceptors characterized in this study (21). The question of whether P4 purinoceptors in CA3 neurons are also antagonized by Ip3.1s is a topic to be investigated.

The Ip3.1s did antagonize the responses elicited by ATP through other purinergic receptors different from the dinucleotide receptor. In this case, Ip3.1, Ip3.2, and Ip3.3 antagonized the Ca2+ response induced by ATP with IC50 values in the micromolar range. Although Ip3.1 and Ip3.2 were not very useful in discriminating between the P4 and P2×x purinoceptors, this was not the case for Ip3.3. Ip3.3 seems to be a good candidate for a dinucleotide receptor antagonist because it is 6000 times more effective on the P4 purinoceptor than on the ATP receptor. This result could be at the origin of new pharmacological tools in the nucleotide receptor field. It is, nevertheless, necessary to take into consideration the features of the presynaptic model in which these experiments were performed.

Ap4A activity on metabotropic P2 purinoceptors have been reported in many tissues (4, 22). The human P2×x purinoceptor is extremely sensitive to Ap4A, and this dinucleotide has been suggested to be a physiological regulator of this receptor (23). Also, receptors for Ap6As described in heart (24–26) and mouse brain (6, 21) would be good models in which Ip3 compounds could be assayed to establish pharmacological criteria for the classification of the dinucleotide receptors.

It is well known that Ap6As are inactivated by the action of high affinity ectodinucleotide hydrolases (27, 28). Based on the results of the current study, possible Ip3.1 formation at the extracellular level by an unidentified adenylate deaminase cannot be ruled out. Further studies are necessary to demonstrate this possibility and its physiological relevance.

In summary, deamination products of Ap6As should be considered when studying the nucleotide receptors. The selectivity of Ip3.1 in antagonizing the dinucleotide receptor suggests that this substance could be an appropriate compound with which to identify P4 purinoceptors in biological systems.

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### References


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