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Short communication

Dopamine and 5-hydroxytryptamine selectively potentiate neuronal type ATP receptor channels

Ken Nakazawa *, Yasuo Ohno

Division of Pharmacology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158, Japan

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Abstract

Dopamine and 5-hydroxytryptamine have been shown to facilitate a cationic current activated by extracellular ATP in rat pheochromocytoma PC12 cells. Effects of these and other modulators were examined by expressing ATP receptor channels in *Xenopus* oocytes using cDNAs from rat vas deferens ('$P2x_1$-purinoceptor channels') and PC12 cells ('$P2x_2$-purinoceptor channels'). Dopamine and 5-hydroxytryptamine (10 and 100 μM) facilitated the ATP-activated current mediated through $P2x_2$-purinoceptor channels, but not the current through $P2x_1$-purinoceptor channels. Adenosine (1 μM) facilitated the current through both $P2x_1$- and $P2x_2$-purinoceptor channels. Cd^{2+} (1 mM) as well as Zn^{2+} (10 μM) selectively potentiated the current through $P2x_2$-purinoceptor channels. The results suggest that (1) the facilitation by dopamine and other modulators also occurs in recombinant ATP-receptor channels, and (2) the selective facilitation by dopamine, 5-hydroxytryptamine and divalent cations of $P2x_2$-purinoceptor channels is attributed to some structural difference of the channels from $P2x_1$-purinoceptor channels.

Keywords: ATP receptor channel; Dopamine; 5-HT (5-hydroxytryptamine, serotonin); Recombinant channel; *Xenopus* oocyte

1. Introduction

Extracellular ATP has been shown to act as a fast neurotransmitter by activating non-selective cation channels (see reviews, Edwards and Gibb, 1993; Surprenant et al., 1995). The ATP-activated channels have been cloned from both neuronal cells (rat pheochromocytoma PC12 cells; Brake et al., 1994) and smooth muscle cells (rat vas deferens; Valera et al., 1994). Although the cloned ATP-activated channels appear to be channel-forming receptors (ATP receptor channels; classically, P_{2x}-purinoceptors), their structures deduced from amino acid sequences are quite different from the motif for the so-called 'ligand-gated channel superfamily' including nicotinic acetylcholine receptors and ionotropic glutamate receptors. Thus, ATP receptor channels have been classified into a novel channel family.

Various compounds including endogenous substances have been reported to modulate the ATP receptor channels in PC12 cells. Dopamine (Inoue et al., 1992; Nakazawa et al., 1993) and 5-hydroxytryptamine (Nakazawa et al., 1994b) facilitated an inward current activated by ATP. Adenosine exhibited both inhibition and facilitation on the ATP-activated current (Inoue et al., 1994). Zn^{2+} potentiated the ATP-evoked responses in these cells (Koizumi et al., 1995), as it did in other mammalian peripheral neurons (Clouse et al., 1993; Li et al., 1993). In the present study, we examined (1) whether the modulations by these compounds are reproducible in ATP receptor channels expressed in *Xenopus* oocytes using cDNA cloned from PC12 cells, and (2) whether the compounds also affect the channels expressed using cDNA cloned from rat vas deferens.

2. Materials and methods

cDNAs encoding ATP receptor channels of PC12 cells (Brake et al., 1994; the Genbank entry U14414) and rat vas deferens (Valera et al., 1994; the EMBL submission X80477) were kindly supplied by Dr. T. Brake of the University of California, San Francisco

* Corresponding author. Tel.: +81-3-3700-1141 ext. 348; fax: +81-3-3707-6950.

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and Dr. G. Buell of Glaxo Institute for Molecular Biology, respectively. The cDNA of PC12 cells has been cloned into pcDNA1/AMP (Invitrogen) (this plasmid has been termed pP2XRI), and that of rat vas deferens has been cloned into pBKCMV (Stratagene). In this report, the channels expressed using cDNA from rat vas deference and those from PC12 cells are called $P2x_1$- and $P2x_2$-purinoceptor channels, respectively, according to the classification by Abbracchio and Burnstock (1994). Procedures for expression of the channels and recordings of membrane currents were basically the same as those utilized for nicotinic receptor channels in our previous report (Nakazawa et al., 1994a). The plasmids were linearized by *Not*I (Toyobo, Osaka, Japan), and sense fragments of RNA were transcribed using T3 ($P2x_1$) or T7 ($P2x_2$) RNA polymerase (Wako, Osaka, Japan). Defolliculated oocytes were injected with the synthesized RNA, and maintained at 18°C in ND96 solution containing (mM) NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, Hepes 5 (pH 7.5 with NaOH) supplemented with 0.01% gentamycin for 3–6 days. Membrane currents were measured by two microelectrode voltage-clamp methods at room temperature (25–27°C). Oocytes were placed in an experimental chamber of about 0.2 ml filled with ND96 solution. ATP and other drugs were applied by superfusion at a constant flow rate of 0.2–0.5 ml/s. ATP was applied for 4–5 s, every 3 min for $P2x_1$-purinoceptor channels and every 1 min for $P2x_2$-purinoceptor channels. These application intervals, which had been determined from preliminary experiments, were long enough to avoid desensitization to ATP upon repetitive applications. The concentration of ATP was 100 nM for $P2x_1$-purinoceptor channels and 30 μM for $P2x_2$-purinoceptor channels. These concentrations are one-third to half of EC_{50} of ATP for the corresponding types of channels (Valera et al., 1994; Brake et al., 1994), and suitable for observation of potentiation of ionic currents through the channels (see 'Results', and Inoue et al., 1992; Nakazawa et al., 1993, 1994b).

Drugs used are ATP (adenosine 5'-triphosphate disodium salt; Sigma, St. Loius, MO, USA), dopamine hydrochloride (Sigma), 5-hydroxytryptamine creatine sulphate complex (Sigma) and adenosine (Sigma). Other chemicals were of reagent grade.

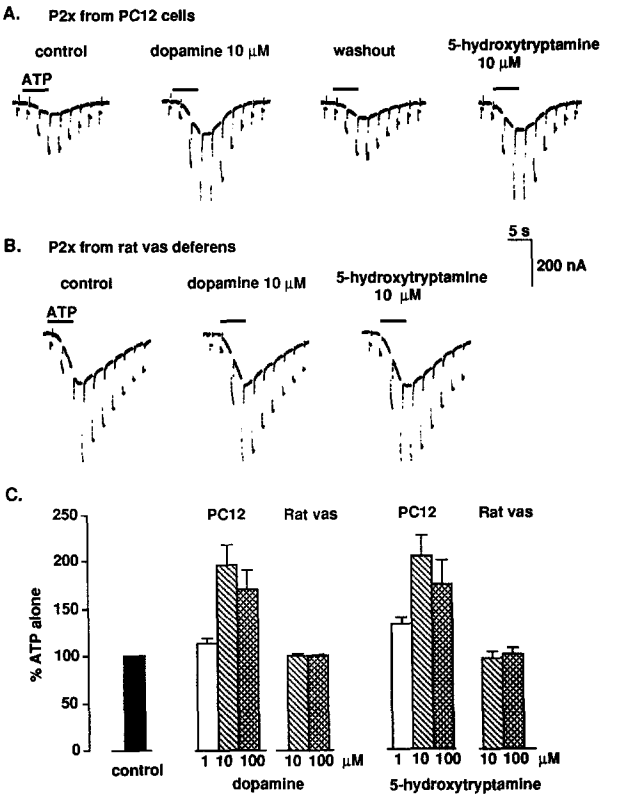

Fig. 1. Effects of dopamine and 5-hydroxytryptamine on recombinant ATP receptor channels in *Xenopus* oocytes. The oocytes were held at −50 mV and hyperpolarized to −100 mV for 200 ms, every 2 s. A: Facilitation by dopamine (10 μM) and 5-hydroxytryptamine (10 μM) of an inward current activated by 30 μM ATP in oocytes expressing ATP receptor channels cloned from PC12 cells ($P2x_2$-purinoceptor channels). Current traces obtained from an oocyte upon four successive applications of ATP were shown. B: Lack of influence of dopamine or 5-hydroxytryptamine on an inward current evoked by 100 nM ATP in an oocyte expressing ATP receptor channels obtained from rat vas deferens ($P2x_1$-purinoceptor channels). C: Summarized data. The amplitude of the ATP-activated current at −50 mV in the presence of various concentrations of dopamine and 5-hydroxytryptamine, obtained as in A or B, was normalized to the current amplitude just before the drug application. Each column and bar represents mean and S.E. from 4–7 oocytes tested.

3. Results

Fig. 1A illustrates inward currents activated by ATP (30 μM) in oocytes expressing $P2x_2$-purinoceptor channels. Dopamine (10 μM) facilitated the ATP-activated current (second panel), and the current was readily returned to the control level when dopamine was removed (third panel). The ATP-activated current was also facilitated by 10 μM 5-hydroxytryptamine (fourth panel). The current facilitation by 5-hydroxytryptamine was also reversible (not shown). A higher concentration of these compounds (100 μM) did not further facilitate the ATP-activated current, and a lower concentration of dopamine or 5-hydroxytryptamine (1 μM) did not facilitate the current (Fig. 1C). The current maximally activated by 300 μM ATP was not facilitated (the current amplitude in the presence of 10 μM dopamine or 10 μM 5-HT was 98.2 ± 2.6% (n = 5) or 93.3 ± 6.2% of control (n = 4), respectively (mean ± S.E.)). These properties are in common with the facilitation observed in PC12 cells (Inoue et al., 1992; Nakazawa et al., 1993, 1994b). In contrast to $P2x_2$-purinoceptor channels, neither dopamine nor 5-hydroxytryptamine

(10 and 100 μM) affected the ATP-activated current through $P2x_1$-purinoceptor channels (Fig. 1B and C).

Unlike dopamine or 5-hydroxytryptamine, adenosine (1 μM) facilitated the ATP-activated current through both $P2x_1$- and $P2x_2$-purinoceptor channels (Fig. 2A and B). At a higher concentration (100 μM), adenosine, however, facilitated the ATP-activated current only through $P2x_2$-purinoceptor channels (Fig. 2B). Zn^{2+} (10 μM) dramatically augmented the current through $P2x_2$-purinoceptor channels, as has been demonstrated by Brake et al. (1994), but it did not affect the current through $P2x_1$-purinoceptor channels (Fig. 2D). A higher concentration of Zn^{2+} (100 μM) did not affect the current through $P2x_1$-purinoceptor channels either ($n = 2$). It was found recently that Cd^{2+}, like Zn^{2+}, exhibited facilitatory effects on cellular responses elicited by ATP in PC12 cells (Ikeda et al., 1995). Such a facilitatory effect of Cd^{2+} (1 mM) was also observed with recombinant $P2x_2$-purinoceptor channels (Fig. 2C and D). Cd^{2+} (1 mM) did not affect $P2x_1$-purinoceptor channels (Fig. 2D). As Cd^{2+} is a well-known blocker of cation-permeable channels (Kass and Osipenko, 1994), it is speculated that the facilitation of $P2x_1$-purinoceptor channels might have been masked by such channel block. From this point of view, we tested a lower concentration (100 μM) of Cd^{2+}, but the current through $P2x_1$-purinoceptor channels was not affected either ($n = 2$).

4. Discussion

The present study has demonstrated that potentiation by various substances, including neurotransmitters, of ATP receptor channels in PC12 cells also occurs in recombinant channels expressed in *Xenopus* oocytes. The potentiation by dopamine and 5-hydroxytryptamine as well as Zn^{2+} and Cd^{2+} was restricted to $P2x_2$-purinoceptor channels, but, in contrast, the potentiation by adenosine was observed with both $P2x_1$- and $P2x_2$-purinoceptor channels.

Dopamine and 5-hydroxytryptamine facilitated recombinant $P2x_2$-purinoceptor channels at concentrations similar to those for the current facilitation in PC12 cells (Nakazawa et al., 1993, 1994b). On the other hand, the concentration dependence of the current facilitation by adenosine in the recombinant of $P2x_2$-purinoceptor channels was different from that in native channels in PC12 cells. The ATP-activated current was inhibited by adenosine at 1 μM, and facilitated at 100 μM in PC12 cells (Inoue et al., 1994) while the facilitation was observed at both concentrations

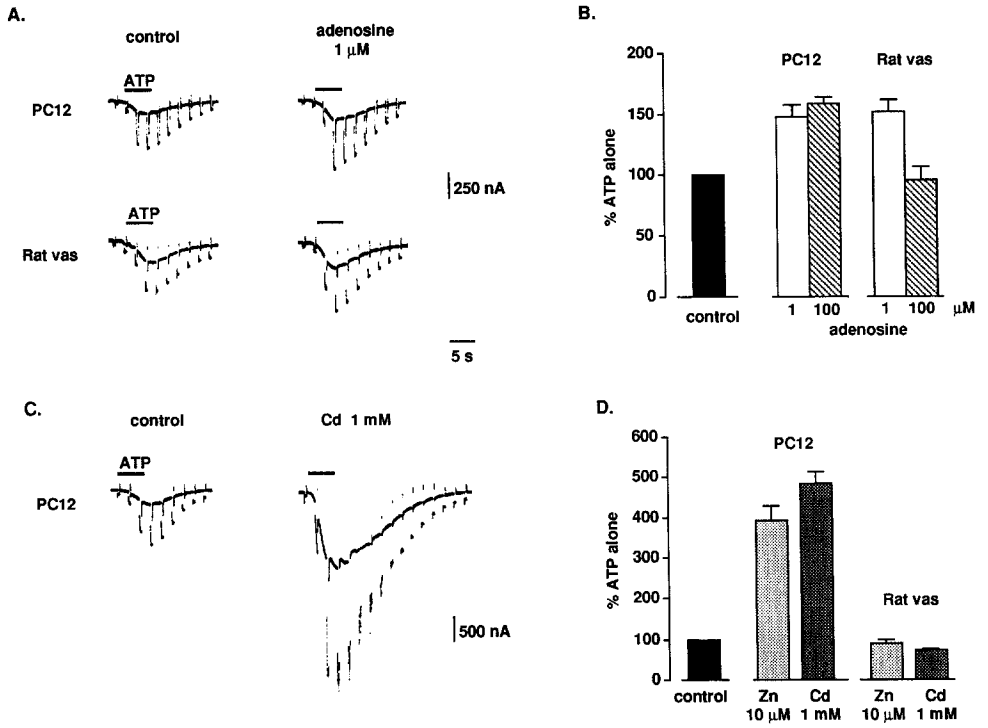

Fig. 2. Effects of adenosine (A, B) and Zn^{2+} and Cd^{2+} (C, D) on recombinant ATP receptor channels expressed in *Xenopus* oocytes. Experimental protocols and assessment of data were the same as in Fig. 1. A, B: Effects of adenosine. A: Facilitation by 1 μM adenosine of inward currents through the channels cloned from PC12 cells ($P2x_2$-purinoceptor channels; upper traces) or those from rat vas deferens ($P2x_1$-purinoceptor channels; lower traces). B: Summarized data for effects of 1 and 100 μM adenosine. Each column and bar represents mean and S.E. from 4–6 oocytes. C, D: Effects of Zn^{2+} and Cd^{2+}. C: Facilitation by 1 mM Cd^{2+} of an inward current through the channels cloned from PC12 cells ($P2x_2$-purinoceptor channels). D: Summarized data. Each column and bar represents mean and S.E. from 4–5 oocytes.

with the recombinant $P2x_2$-purinoceptor channels (Fig. 1). Some cellular component existing in PC12 cells may modify the reactivity to adenosine.

The facilitation by dopamine, 5-hydroxytryptamine of the recombinant channels, as well as that by Zn^{2+} and Cd^{2+}, indicate that these compounds directly modulate through binding sites on channel protein itself because the channels were solely expressed (i.e., without expression of additional components) in the present study. It should, however, also be noted that at present we cannot completely exclude the contribution of intrinsic cellular components in *Xenopus* oocytes. In any case, as our results were obtained using expressed channels, the different reactivity to dopamine and other substances between $P2x_2$-purinoceptor channels can be attributed to a diversity in their structures. As similarity in amino acid sequences of these two subclasses of purinoceptor channels was estimated to be 54% (Surprenant et al., 1995), some structure particular to $P2x_2$-purinoceptor channels may account for the selective facilitation. Determination of structural requirement for the facilitation remains to be clarified, and such clarification will be able to provide a clue to the significance of structural diversity between the subclasses of ATP receptor channels. Finally, the facilitation of neuronal type purinoceptor channels reported here may be physiologically significant because 5-hydroxydopamine and dopamine are well-known neurotransmitters and ATP has also been shown to act as an excitatory neurotransmitter (Edwards and Gibb, 1993) in the central nervous system. The facilitation by Zn^{2+} may also play a physiological role because Zn^{2+}-containing neurons have been identified (Frederickson, 1989), and their interaction with ATP receptor channels has been suggested (Seguela et al., 1995).

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