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Functional expression of 2-amino-4-phosphonobutyrate (APB) receptors in *Xenopus laevis* oocytes by injection of poly(A)$^+$ RNA from quail brain

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Abstract

The glutamate analogue 2-amino-4-phosphonobutyrate (APB) is known to activate a subtype of metabotropic glutamate receptor in the central nervous system, including the retina. In the present study, APB receptors were studied using the *Xenopus* oocyte expression system. No endogenous APB sensitivity was detected in control oocytes. In contrast, microinjection of mRNA, extracted from quail brain, into *Xenopus* oocytes resulted in the functional expression of APB receptors after 3–5 days incubation. Application of 50 μM–1 mM APB to injected oocytes voltage clamped at a holding potential of −60 mV produced a sustained outward current which was associated with a significant *decrease* in membrane conductance; the reversal potential was around −11 mV. The response to APB was dose-dependent and non-desensitizing. This is the first demonstration of the expression of a conductance-decreasing receptor mechanism in *Xenopus* oocytes.

Key words: 2-Amino-4-phosphonobutyrate (APB); Glutamate; Receptor; Quail brain; *Xenopus* oocyte; mRNA; Microinjection

1. Introduction

Glutamate, a major excitatory neurotransmitter in the vertebrate central nervous system (CNS), acts on a variety of ionotropic and metabotropic receptors [1,2,5,8,9,12–15,18]. The glutamate analogue 2-amino-4-phosphonobutyrate (APB) activates a subtype of metabotropic receptor to produce a variety of cellular responses. In brain and spinal cord, APB receptors are pre-synaptic and mediate synaptic antagonism by suppressing the release of glutamate [1,3]. In the retina, APB activates conductance-decreasing post-synaptic receptors present upon centre-depolarizing (i.e. ON-) bipolar cells [5,6,20–22,24] and luminosity (H1 type) horizontal cells [5,19,21,25,27]. The bipolar cell receptor is responsible for separating the retinal signals into 'ON' vs. 'OFF' pathways and thus plays an important role in the visual process [4,10,17,23,26]. A retinal cDNA clone (mGluR6) was expressed in Chinese hampster ovary cells and shown to code for an APB receptor which appeared to be associated with ON-bipolar cells and negatively linked to adenylate cyclase; however, its electrophysiology was not investigated [14]. In the present study, APB-sensitive, conductance-decreasing receptors were found by functional expression in *Xenopus* oocytes following microinjection of poly(A)$^+$ RNA extracted from quail brains.

2. Materials and methods

The procedures used were as described previously [6,7]. Whole brains were removed from adult quail and frozen immediately in liquid nitrogen. Total RNA was extracted from 10 brains by the guanidinium isothiocyanate method. Poly(A)$^+$ RNA was purified on oligo d(T) cellulose and concentrated by ethanol precipitation. The poly(A)$^+$ RNA was resuspended in diethyl pyrocarbonate-treated sterile water at a concentration of 1 mg/ml. In order to obtain oocytes, lobes of ovary were dissected from female *Xenopus laevis* under ethyl-*m*-aminobenzoate (MS222) anaesthesia. Stage V–VI oocytes were manually teased apart and left for several hours before RNA injection. Injection pipettes were pulled from thin-walled glass capillaries and their tips broken to 10–15 μm and silanized before use. Each oocyte was injected with 40–60 nl of RNA and groups of 5–6 oocytes were then placed in modified Barths' medium at 20°C in an incubator until use. The incubation medium was changed daily and experiments commenced from the third day after the microinjection. Oocytes were tested electrophysiologically by two-electrode voltage clamp using micro-electrodes filled with 2.5 M KCl and having resistances of 1–3 MΩ. Frog Ringer (in mM: NaCl 115, KCl 2.5, $CaCl_2$ 1.8, HEPES 5; pH 7.3) was continuously perfused at a flow rate of 3–4 ml/min. APB was dissolved at a working concentration of 1 mM in the frog Ringer and was prepared daily. Electrophysiological data were analysed as means ± S.E.M.; experimental numbers refer to different oocytes.

3. Results and discussion

Oocytes were pharmacologically screened initially at a holding potential of −60 mV. Uninjected (i.e. control) oocytes did not respond to 1 mM glutamate or its agonists, APB and kainate. On the other hand, application of APB to RNA-injected oocytes produced a response that comprised an outward current that was smooth and sustained, and preceeded by a transient inward component (Fig. 1). The response was reversed slowly by washing with the control Ringer solution. Application of glu-

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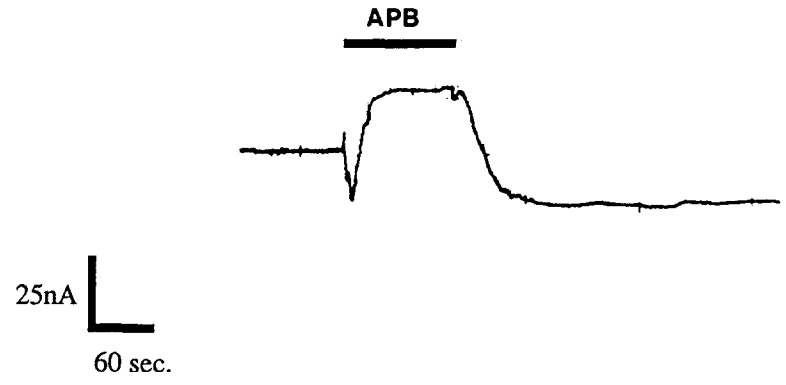

Fig. 1. Effect of 1 mM APB on the membrane current in an oocyte microinjected with about 50 nl of poly(A^+) RNA (1 mg/ml) from quail brain. The oocyte was voltage clamped at a holding potential of −60 mV. The duration of application of APB is indicated by the horizontal bar above the response trace. A smooth sustained outward membrane current followed a fast transient inward membrane current. The response was gradually reversed by washing with normal Ringer solution.

tamate (1 mM) produced similar currents but with much smaller amplitudes; equimolar kainate was very effective generating smooth sustained inward currents of much bigger amplitude (not illustrated).

The APB-induced response was studied further and found to have the following characteristics. It was dose-dependent in the concentration range from 50 μM to 1 mM and did not desensitize during prolonged or repeated applications of the agonist. Current–voltage (*I–V*) relationships were measured before, during the steady-state (i.e. sustained phase) and after the application of APB (Fig. 2). Comparison of the membrane conductances before and during the APB response revealed a consistent *decrease*, on average from 2.2 ± 0.3 mS to 1.9 ± 0.3 mS (n = 8). A paired t-test performed for these values showed that the difference was highly significant ($P<0.0005$). The average value of the reversal potential was −10.9 ± 4.6 mV, consistent with the involvement of a non-specific cation channel. The general excitatory amino acid antagonist kynurenate (1 mM) blocked the APB response, whilst the inhibitory amino acid $GABA_A$ receptor/chloride channel blocker picrotoxin (100 μM) had no effect (not illustrated).

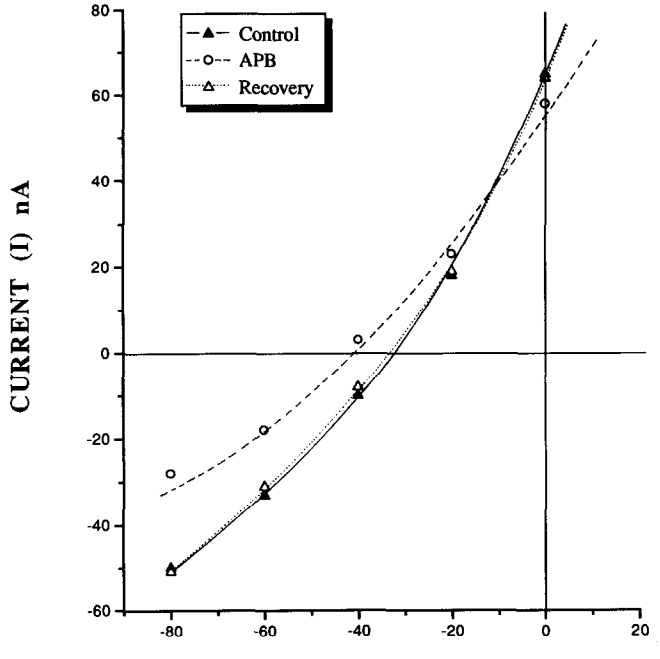

Fig. 2. Typical current–voltage (*I–V*) relationships for a single oocyte, measured before (▲, continuous line), during (△, dashed line) and after (○, dotted line) application of 0.5 mM APB. The data show that APB decreased the membrane conductance (from 4.0 to 3.7 nS in this cell, measured from the linear portions of the *I–V* curves). The reversal potential corresponds to −11 mV. The effect of APB was fully reversible.

The results of the present experiments demonstrate that poly(A)$^+$ RNA extracted from quail brains and microinjected into *Xenopus* oocytes results in the expression of electrophysiologically functional, APB-sensitive, conductance-decreasing receptors, probably gating a cationic channel, that were absent from the plasma membrane of the native oocytes. This is the first demonstration of the expression of such a receptor in *Xenopus* oocytes. In the CNS, APB activates a subtype of metabotropic glutamate receptor [6,13,15]. The functional characteristics of APB receptors is best understood, however, from work on the vertebrate retina where ON-centre bipolar cells, and possibly horizontal cells, respond to APB post-synaptically via stimulation of a G-protein. The latter activates a phosphodiesterase leading to a decrease in the concentration of the cyclic nucleotide second messenger, cGMP [4,17,19]. In turn, the reduction in cGMP results in suppression of the cGMP-activated cation conductance [15,17].

The present results are consistent with such a scheme. However, it is not clear whether the cation conductance-decreasing APB receptors expressed in oocytes are linked

to a G-protein activating a phosphodiesterase. We would also note that the complex waveform of the APB-induced current that we recorded in the mRNA-injected oocytes could be indicative of the presence of multiple APB receptors in quail brain. Further expression studies are required to elicudate these aspects and to reveal which other proteins are involved in the action of APB on injected oocytes. This approach could further elucidate the functional and molecular characteristics of APB receptors in the CNS and retina.

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