## Short communication

## Regulation of Beta-Adrenergic Response in Xenopus laevis Oocytes

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It has been demonstrated that Xenopus laevis oocytes are sensitive to catecholamines. The oocyte response is characterized as being of beta adrenergic nature and is electrophysiologically measured as a transient increase in potassium conductance (hyperpolarizing current in holding potential -40 mV) (VanRenterghem et al.1985).The aim of this paper was to study the decline of the oocyte response observed during continuous isoprenaline application.

The experiments were performed on oocytes from the ovary of Xenopus laevis prepared manually. All experiments were carried out with oocytes at stages V and VI (Dumont 1972). The oocytes were stored in a modified sterile Barth's medium at 15 °C (Stinnakre and VanRenterghem 1986). The two-microelectrodes voltage clamp method was used for electrophysiological recordings. The membrane potential of the oocytes was clamped at -40mV during all experiments. One oocyte per experiment was placed into a chamber continuously perfused with Ringer saline at 3ml/min. Drug solutions were applied by bath perfusion after adjusting pH to 7.2-7.4, and/or by pressure injection into the cell through a third micropipette with a tip diameter less than 10  $\mu$ m.

The oocyte response to isoprenaline is characterized by a latency of the response (approximately 30 s) followed by a fast increase of the outward current (Fig. 1*a*). This current is the result of a change in potassium permeability (Van-Renterghem et al. 1985). The time to peak was reached within 100 s. With isoprenaline concentrations exceeding  $10^{-7}$  mol/l, the time to peak was shorter. The current decreased in the continuous presence of the drug.

The effect of isoprenaline on Xenopus laevis oocyte is mediated by beta adrenergic receptor, adenyl cyclase system and cAMP-dependent changes of potassium conductance (VanRenterghem et al. 1985). The decline in K<sup>+</sup> permeability after a transient increase during continuous isoprenaline treatment may be due to:

1) fast inactivation of K<sup>+</sup> ion channels,



Figure 1. Potassium currents induced by isoprenaline and phosphodiesterase inhibitors IBMX and theophylline in 5 oocytes from 5 donors. a: Outward-hyperpolarizing currents induced by  $5.10^{-7}$  mol/l isoprenaline (ISO) and isoprenaline +  $5.10^{-3}$  mol/l theophylline (THE); b: Current evoked by simultaneous application of isoprenaline+theophylline (0.5- $5.10^{-3}$  mol/l); c: The effect of another phosphodiesterase inhibitor isobuthylmethylxanthine (IBMX,  $5.10^{-4}$  mol/l) during prolonged application of isoprenaline; d: The oocyte response to a gradual increase of isoprenaline concentrations from  $5.10^{-8}$  to  $5.10^{-6}$  mol/l. The interval was 25min.; e: Gradual increase of isoprenaline concentrations followed by a treatment with  $10^{-6}$  mol/l isoprenaline+1BMX  $5.10^{-4}$  mol/l. Holding potential was -40 mV in all tests.

- beta receptor desenzitization, its phosphorylation and uncoupling from adenylyl cyclase system (Sibley et al. 1986),
- an enhanced cAMP degradation by cAMP-specific phosphodiesterases present in oocytes (Allende et al. 1977, Allende and Plaza 1987).

The first possibility is improbable. cAMP injection into the oocyte induced a  $K^+$  current with a desenzitization smaller than that of the  $K^+$  current evoked by isoprenaline (Fig. 2e). Isoprenaline evoked  $K^+$  current (Fig. 2d,e) whereas cAMP-activated  $K^+$  current decreased. In some cases the decay time of cAMP-induced



Figure 2. Potassium currents induced by isoprenaline, adenosine and cAMP. a: The oocyte response to  $10^{-6}$  mol/l isoprenaline (ISO), 30 min. before sustained applications of adenosine; b: Sustained application of  $10^{-5}$  mol/l adenosine (ADE) with simultaneous applications of adenosine+isoprenaline at 6; 35; 65 and 95 min.; c: Sustained treatment of another oocyte with  $2.10^{-7}$  mol/l isoprenaline and simultaneous applications of isoprenaline  $+10^{-5}$  mol/l adenosine at 4; 33; and 60 min., control adenosine response after 30 min.; d: The oocyte response to  $10^{-6}$  mol/l isoprenaline, 30 min. before an injection of cAMP into the oocyte; e: Injection of 13 pmol cAMP/oocyte and response to isoprenaline. Holding potential was -40 mV in all experiments.

 $K^+$  current was several minutes. This long decay time was a result of a slowlier inactivation of  $K^+$  channels and/or the large amount of cAMP injected (above 10 pmol/oocyte). Measureable current was evoked by 0.15 pmol cAMP/oocyte (Lotan et al. 1985). It may be supposed that cAMP injection and isoprenaline treatment were mediated by the same molecular mechanism (Stinnakre and VanRenterghem 1986; Lotan et al. 1985; VanRenterghem et al. 1985). The decrease of the K<sup>+</sup> current evoked by cAMP injection was slowlier than that of the K<sup>+</sup> current evoked by isoprenaline; hence, it may be suggested that the contribution of fast inactivation of K<sup>+</sup> channels to the fast inactivation of the isoprenaline response observed was negligible.

Rapid desenzitization of beta-adrenergic receptors would be expected to induce receptor uncoupling from the adenylyl cyclase system followed by a decline of K<sup>+</sup> current. However, simultaneous treatment with isoprenaline and an inhibitor of phosphodiesterases theophylline, during the phase of resting current in response to sustained isoprenaline application, produced a response similar to the isoprenaline effect (Fig. 1a, b, c). The phosphodiesterase inhibitors theophylline and IBMX are derivatives of xanthine, as is caffeine, which in some tissues induces calcium release from internal sources (Kuba and Nishi 1976). The possibility that the inhibitors activated Ca<sup>2+</sup> dependent K<sup>+</sup> current is not likely. The treatment of oocytes with an inhibitor by itself never induced  $K^+$  current; a simultaneous application with isoprenaline however potentiated cAMP dependent K<sup>+</sup> current (VanRenterghem et al. 1985; Lotan et al. 1985; Mori et al. 1989). The response of oocytes to acetylcholine is mediated by muscarinic receptor and by the increasing of the intracellular level of calcium (Dascal 1987). Calcium ions activated Ca<sup>2+</sup> dependent Cl<sup>-</sup> current and theophylline inhibited the acetylcholine response (Dascal and Cohen 1987). The inhibitory effect of theophylline was probably mediated by inhibition of Ca<sup>2+</sup> release from internal pool similarly as in PC12 cells (Vicentini et al. 1986).

As shown in Fig. 1a, b, c K<sup>+</sup> current reappeared upon addition of phosphodiesterase inhibitors to isoprenaline; under this condition, oocyte response to isoprenaline is zero. The course of this "new" K<sup>+</sup> current was similar to that induced by isoprenaline. The peak was reached within 100s with subsequent decline of the  $K^+$  current. Another  $K^+$  current was evoked by a higher concentration of the inhibitor (Fig. 1b). If a higher concentration of the inhibitor was not used the  $K^+$ current disappeared (Fig. 1c). The ratio of the current amplitudes induced by isoprenaline  $(5.10^{-7} \text{mol/l})$  plus theophylline  $(5.10^{-3} \text{mol/l})$  and isoprenaline was 2.3  $\pm$  S.D.0.4 (n=36), respectively. The decline of K<sup>+</sup> permeability can be explained by an enhanced activity of phosphodiesterases after activation of beta adrenergic receptors. An increase of cAMP level stimulated the activity of cAMP dependent protein kinase A which also phosphorylated the phosphodiesterases. Thus, when activated phosphodiesterases produced negative back coupling (Corbin et al. 1988). The decline of the inhibitor-induced current and its reevocation by a higher concentration of the inhibitor suggested that increased cAMP levels further stimulated phosphodiesterases. This assumption was supported by experiments with gradually raised isoprenaline concentrations (Fig. 1d, e). After oocyte reacted to low a concentration of isoprenaline, the higher isoprenaline concentrations did not induce a measureable current. K<sup>+</sup> current could be evoked by phosphodiesterase inhibitor IBMX or when some time was allowed between the applications. The possibility of a gradual activation of phosphodiesterases by accumulating cAMP has been suggested by experiments in which isoprenaline was applied simultaneously with the purinergic agonist adenosine (Fig. 2a, b, c). Purinergic receptors

are present in *Xenopus laevis* oocytes and their properties are similar to those of beta adrenergic receptor (Lotan et al. 1985; Stinnakre and VanRenterghem 1986). Fig. 2a, b, c illustrates the results of simultaneous treatment with adenosine and isoprenaline. After 65 min the isoprenaline response was comparable with the control response. It suggested a high activity of phosphodiesterases in the presence of permanent cAMP production: the adenosine response was zero and desenzitization of beta adrenergic receptors was insignificant. In summary, our results suggest that regulation of oocyte response to isoprenaline was mediated by raised of phosphodiesterase activity.

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