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Activation of a common effector system by different brain neurotransmitter receptors in *Xenopus* oocytes

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Xenopus oocytes possess 'native' muscarinic receptors, which give rise to oscillatory chloride currents; similar responses are elicited by activation of foreign receptors to serotonin, glutamate and noradrenaline, expressed in oocytes after injection of messenger RNA from rat brain. When low concentrations of two agonists are applied together, the combined response is greater than would be expected from the sum of the responses to each agonist applied alone. Potentiation of acetylcholine by serotonin is blocked by the serotonin antagonist methysergide; conversely, the potentiation of serotonin by acetylcholine is blocked by the muscarinic antagonist atropine. This indicates that each agonist acts on a distinct receptor. The interactions between serotonin, acetylcholine and other agonists provide further evidence that the different receptors may all 'link in' to a common receptor–channel coupling system, in which phosphoinositide metabolism and calcium liberation lead to the opening of chloride channels in the oocyte membrane.

Introduction

Many oocytes of *Xenopus laevis* possess muscarinic receptors which, when activated by acetylcholine (ACh), give rise to oscillatory chloride membrane currents (Kusano *et al.* 1977, 1982). In addition to these 'native' receptors, a variety of other neurotransmitter receptors can be induced in oocytes, by injecting them with messenger RNA (mRNA) from brain and other sources. Several of these foreign receptors, including those to serotonin (Gundersen *et al.* 1983), glutamate (Gundersen *et al.* 1984; Houamed *et al.* 1984), noradrenaline (Sumikawa *et al.* 1984*a*), neurotensin and substance P (Parker *et al.* 1986), also give rise to oscillatory chloride currents, like those activated by ACh. Additional similarities between the responses to ACh, serotonin and glutamate include their dependence on intracellular calcium (Parker *et al.* 1985*b*; Dascal *et al.* 1985), the appearance of a transient inward current on hyperpolarization Parker *et al.* 1985*a*), and the appearance of an oscillatory current on cooling (Miledi *et al.* 1987).

The many similarities between the oscillatory responses to the different agonists suggest some common mechanism. However, it was already clear that entirely different types of receptor are involved, because these could be distinguished pharmacologically (Gundersen *et al.* 1984; Sumikawa *et al.* 1984*a*; Parker *et al.*

1986), or by density-gradient centrifugation of the mRNAs which encode their respective receptor proteins (Sumikawa *et al.* 1984*b*; Parker *et al.* 1986). An alternative explanation is that different receptors may share a common link between receptor activation and channnel opening, which we will refer to as receptor–channel coupling, by analogy with excitation–contraction coupling in muscle. Thus, after activation, the different receptors may couple to a common intracellular messenger system, which activates the final chloride-current response. Here we have tested this idea by looking for interactions between responses to different neurotransmitters, as might be expected if they share a common pathway.

Methods

Experiments were made on oocytes of *Xenopus laevis*, which were injected with poly(A)$^+$ mRNA from rat brain to induce the appearance of many types of neurotransmitter receptors. Procedures for extraction of mRNA, injection into oocytes, and electrophysiological recording were as described previously (Miledi & Sumikawa 1982; Miledi 1982). Membrane currents were recorded from oocytes that were voltage clamped at a potential of -60 mV, in response to drugs applied by continuous bath perfusion of Ringer solution at room temperature.

Results

Facilitation between responses to serotonin and acetylcholine

The basic phenomenon is shown in figure 1. Serotonin and ACh were first applied separately to an oocyte injected with rat brain mRNA at doses selected to give small responses. For the oocyte illustrated, serotonin at a concentration of 10^{-7} M gave a peak response of 18 nA, and 3×10^{-7} M ACh a response of 9 nA; while higher concentrations of serotonin (10^{-5} M) and ACh (10^{-4} M) gave responses of, respectively, 720 nA and 930 nA (not illustrated). If the currents elicited by ACh and serotonin were generated independently, the response to the low concentrations of both agonists applied together would be the sum of the response to each drug alone; i.e. 27 nA. However, when they were applied simultaneously, a larger response of 90 nA was observed (figure 1*c*). Thus, the observed response was facilitated by a factor of 3.3 over that expected from a linear summation of the responses.

As well as potentiating the response size, the simultaneous application of ACh and serotonin shortened the latency to onset of the membrane current responses. In the case illustrated, the currents elicited by each drug alone did not become detectable until 3–5 min after their addition to the perfusion fluid (figure 1*a*,*b*) (see also Kusano *et al.* 1977, 1982; Gundersen *et al.* 1983). In contrast, the latency shortened to about 1 min with simultaneous application of ACh and serotonin (figure 1*c*), of which a large part arose from dead time in the perfusion system.

One explanation for the increased response size with simultaneous drug application might be that, instead of each drug acting on a specific receptor, the ACh acted directly on the serotonin receptor, or vice versa. This appears not to be the case, because addition of the specific serotonin antagonist methysergide, together

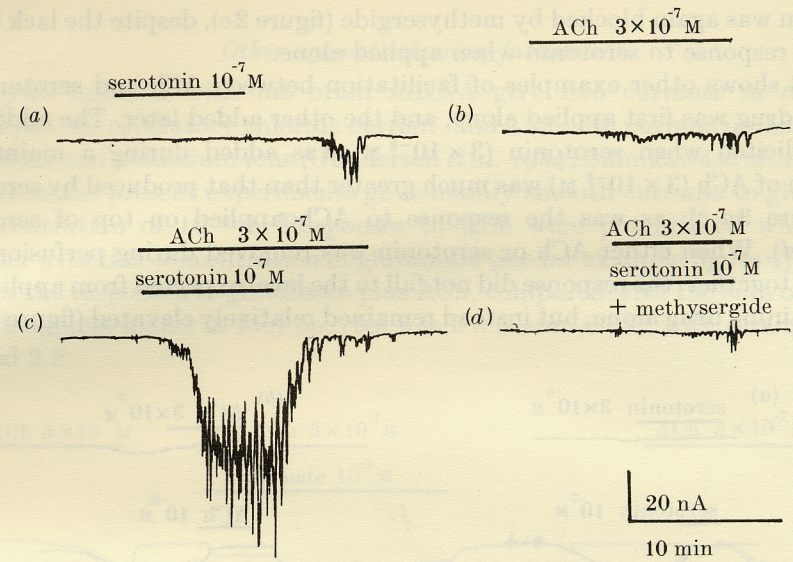

FIGURE 1. Interactions between responses to low doses of acetylcholine and serotonin. In this and subsequent figures, the traces show membrane currents, recorded at a clamp potential of −60 mV, in oocytes injected with mRNA from rat cerebral cortex. Each figure illustrates data from a single oocyte. Inward membrane currents correspond to downward deflections. Drugs were applied in the bathing fluid at the concentration indicated, for the times marked by the bars. (*a*,*b*) Currents elicited by separate applications of serotonin and ACh. (*c*) Response to simultaneous application of ACh and serotonin. (*d*) Blocking of potentiation by methysergide (10^{-6} M), which was applied continuously beginning just after the start of the trace.

with ACh and serotonin, abolished the facilitation, to leave a response of similar size to that obtained with ACh alone (figure 1*d*). Conversely, the potentiation of the response to serotonin by ACh was blocked by atropine, which is known to block the muscarinic ACh receptors in the oocyte (Kusano *et al.* 1977, 1982).

Potentiation was sometimes observed even when the concentration of one, or both, drugs was lowered so that no response was detected when given alone. For example, in figure 2*b* no response was seen to 5×10^{-9} M serotonin, but the current elicited by ACh (3×10^{-7} M) was almost three times greater when applied together with the serotonin (figure 2*b*) rather than alone (figure 2*a*). This facilitatory action

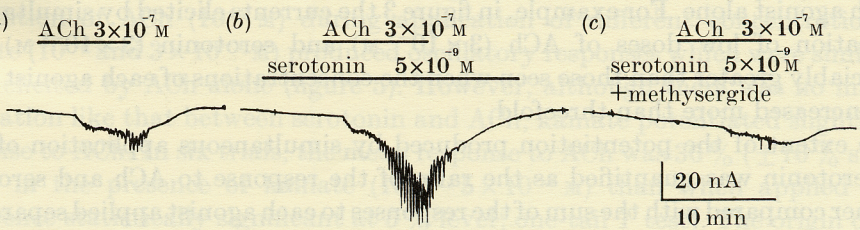

FIGURE 2. Potentiation of response to ACh by a low dose of serotonin, which by itself gave almost no current. In (*c*), methysergide (10^{-6} M) was continuously applied, beginning at the start of the trace.

of serotonin was again blocked by methysergide (figure 2*c*), despite the lack of any detectable response to serotonin when applied alone.

Figure 3 shows other examples of facilitation between ACh and serotonin, in which one drug was first applied alone, and the other added later. The additional response elicited when serotonin (3×10^{-8} M) was added during a maintained application of ACh (3×10^{-7} M) was much greater than that produced by serotonin alone (figure 3*a*,*c*), as was the response to ACh applied on top of serotonin (figure 3*b*,*d*). When either ACh or serotonin was removed during perfusion with both drugs together, the response did not fall to the level expected from application of the remaining drug alone, but instead remained relatively elevated (figure 3*c*, *d*).

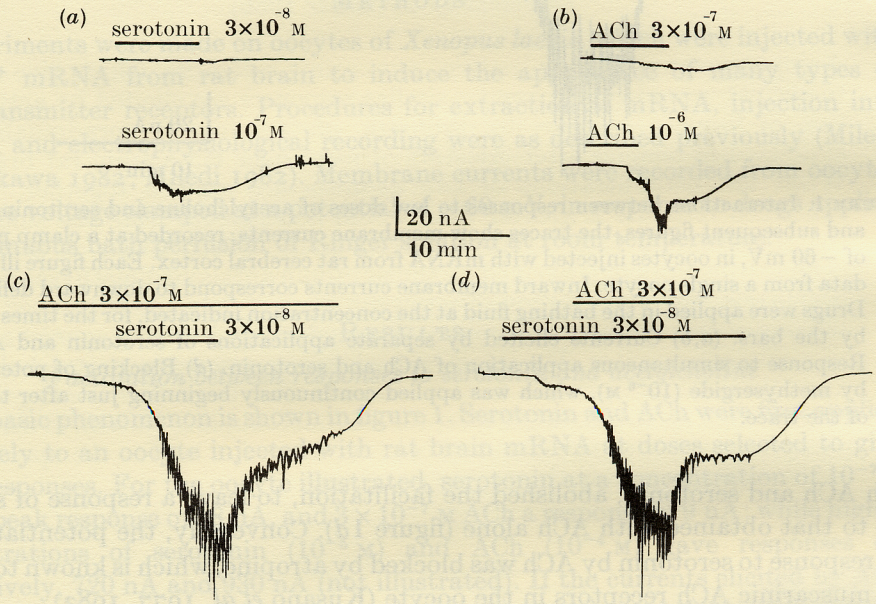

FIGURE 3. Potentiation of serotonin applied during a continued application of ACh, and vice versa. (*a*,*b*) Responses to different doses of serotonin (*a*) and ACh (*b*) applied alone. (*c*,*d*) Potentiation with application of both drugs.

A further point is that the responses produced by ACh and serotonin together could be greater than those produced by comparable increases in concentration of each agonist alone. For example, in figure 3 the currents elicited by simultaneous application of low doses of ACh (3×10^{-7} M) and serotonin (3×10^{-8} M) were appreciably greater than those seen when the concentrations of each agonist alone were increased more than threefold.

The extent of the potentiation produced by simultaneous application of ACh and serotonin was quantified as the ratio of the response to ACh and serotonin together compared with the sum of the responses to each agonist applied separately. This varied considerably, generally being larger for doses of drugs which individually gave very small responses. In nine oocytes the mean ratio was 12.4 (s.e.m. 5.8) with a range betwen 1.6 and 57.

Other agonists showing facilitation

Oocytes injected with rat brain mRNA give two currents in response to glutamate: an oscillatory chloride current, and a smooth current probably carried by sodium and potassium ions (Gundersen *et al.* 1984; Sumikawa *et al.* 1984*b*). The oocytes in the present experiments gave mainly smooth currents to glutamate at a concentration of 10^{-4} M. Responses to ACh were potentiated when applied together with this concentration of glutamate (see for example figure 4). The mean ratio of the responses to glutamate plus ACh, compared with the sum of responses to each agonist alone, in four oocytes was 2.3 (s.e.m. 0.28), with a range between 1.6 and 2.9.

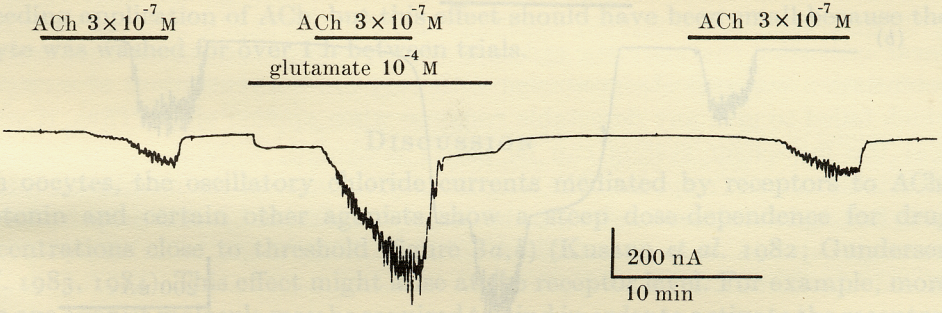

FIGURE 4. Interactions between responses to ACh and glutamate.

Potentiation was also observed between the oscillatory currents elicited by serotonin and noradrenaline. For example, one oocyte gave a response to serotonin (10^{-8} M) plus noradrenaline (10^{-4} M) which was 3.8 times greater than the sum of the responses to each agonist alone.

Kainate and ACh

Kainate receptors induced in the oocyte by injection of rat brain mRNA give rise to a smooth membrane current with short latency, which inverts at a potential of about 0 mV and which is clearly different from the oscillatory choride current activated by ACh or serotonin (Gundersen *et al.* 1984). The kainate-activated channel, therefore, is probably controlled by a mechanism different from that which controls the chloride channels that give rise to the oscillatory currents.

Addition of ACh (10^{-7} M) during application of different concentrations of kainate (10^{-5} and 3×10^{-5} M) produced oscillatory responses which were similar to those elicited by ACh alone (figure 5). However, although there was no marked facilitation like that between serotonin and ACh, kainate potentiated slightly the response to ACh. In six trials, the mean response to ACh was 36% ($\pm$16% s.e.m.) larger in the presence of kainate ($10^{-5}-5\times10^{-5}$ M) than when applied alone (difference statistically significant at 5% level; one-tail T test). The origin of this effect is not yet clear, but it may be related to a kainate-induced influx of calcium into the oocyte, similar to that found at the giant synapse of the squid (Miledi & Stinnakre 1977; Eusebi *et al.* 1985).

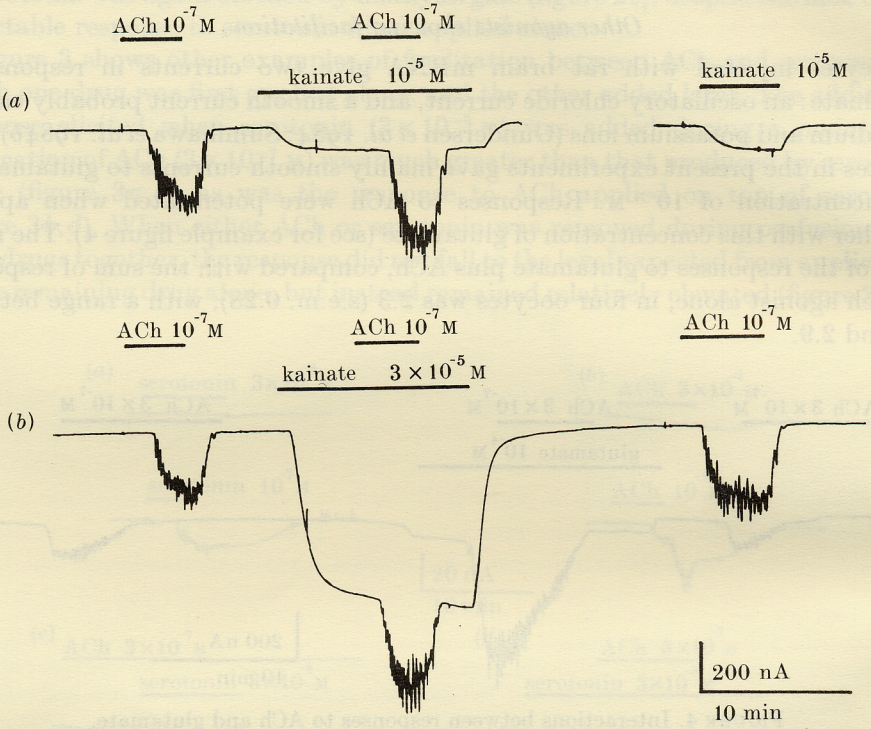

FIGURE 5. Currents elicited by ACh and kainate.

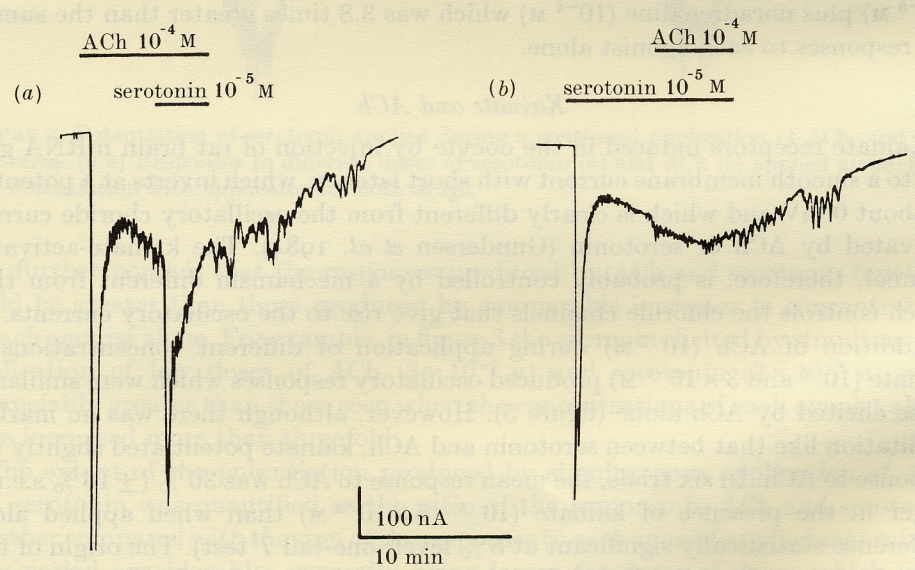

FIGURE 6. Depression of responses by simultaneous application of high concentrations of ACh (10^{-4} M) and serotonin (10^{-5} M). The oocyte was washed for 1.5 h between (*a*) and (*b*). The initial response to ACh in (*a*) went off-scale, reaching a peak current of 1450 nA.

High doses of ACh and serotonin

Application of a high dose of ACh in addition to a high dose of serotonin (and vice versa) gave extra currents which were not larger than those seen with the agonist applied alone, and indeed were often strongly depressed. For example, the oocyte in figure 6 gave an initial 'spike' current of nearly 1.5 μA to ACh (10^{-4} M), followed by a more maintained current of 200–300 nA. However, when ACh was later applied in addition to serotonin (10^{-5} M), it gave an extra current of only about 50 nA. A complication in these experiments was that responses to repeated high doses of ACh or serotonin usually show marked desensitization unless very long times are allowed between applications. Thus, some of the reduction in response to ACh in figure 6*b* might have arisen from desensitization due to the preceding application of ACh, but this effect should have been small because the oocyte was washed for over 1 h between trials.

Discussion

In oocytes, the oscillatory chloride currents mediated by receptors to ACh, serotonin and certain other agonists show a steep dose-dependence for drug concentrations close to threshold (figure 3*a*,*b*) (Kusano *et al.* 1982; Gundersen *et al.* 1983, 1984). This effect might arise at the receptor level. For example, more than one agonist molecule may be required to bind in order to activate the receptor, as with nicotinic ACh and excitatory glutamate receptors (Katz & Theslef 1957; Cull-Candy *et al.* 1981). However, the present results showing potentiation between low doses of agonists suggest a different explanation. Kusano *et al.* (1982) proposed that the chloride channels are opened after the build-up of some substance (second messenger) within the oocyte. Thus, facilitation might arise at some stage in the internal messenger pathway.

The actions of ACh and serotonin in the oocyte almost certainly arise from distinct and specific receptor types that are encoded by different mRNAs (Sumikawa *et al.* 1984*b*) and that can be selectively blocked by different antagonists. Thus, potentiation of the response to ACh by a low dose of serotonin does not occur because both agonists bind to a single receptor type, but instead probably arises from summation in a common pathway activated by the two receptors. This argument is further supported by the results on interactions between ACh and excitatory amino acid receptors. Glutamate receptors expressed in the oocytes give rise to several types of response, including an oscillatory chloride current (Gundersen *et al.* 1984; Sumikawa *et al.* 1984*b*) which is probably mediated via the same messenger pathway as serotonin and ACh receptors. As expected from this, low doses of glutamate potentiated the responses to ACh. On the other hand, we found little facilitation between the oscillatory response to ACh and the smooth current mediated by kainate, which probably involves a very different receptor–channel coupling mechanism (Gundersen *et al.* 1984).

Injections of inositol 1,4,5-trisphosphate ($InsP_3$) into the oocyte generate oscillatory chloride currents which mimic those evoked by agonists such as ACh and serotonin (Oron *et al.* 1985; Miledi *et al.* 1987; Parker & Miledi 1986).

Furthermore, the oscillatory currents are abolished by intracellular injection of the calcium chelating agent EGTA (Parker *et al.* 1985*b*; Dascal *et al.* 1985). Thus, the agonist-evoked currents probably arise through a common internal messenger pathway involving $InsP_3$ and calcium, similar to that proposed for various other cell types (Berridge & Irvine 1984; Hirasawa & Nishizuka 1985; Berridge 1986). It is not yet clear, however, at which stage in this pathway the signals from different receptors converge, or where the interactions between these signals occur. Possibilities include the activation of phospholipase C via GTP-binding proteins, hydrolysis of inositol phospholipid, $InsP_3$-mediated release of calcium from intracellular stores and calcium-dependent activation of membrane chloride channels.

The potentiation seen between low doses of different agonists suggests that one or more stages in the receptor–channel coupling pathway must show a more than linear activation characteristic at low levels of receptor activation. Furthermore, potentiation was still observed even with very low concentrations of serotonin, which failed to give any detectable response when applied alone. Thus, it seems that the messenger system can be partly activated, without giving rise to a detectable current response. The long latency to onset of the membrane current with low agonist doses might also be largely explained by this property of the messenger system. Some intermediate in the pathway might build up slowly, and exceed the threshold level required for channel activation only after several minutes.

In contrast to the behaviour at low agonist concentrations, potentiation was not seen with high concentrations of ACh plus serotonin, and indeed the current was often smaller than would be expected from a linear summation of the responses to each agonist alone. In this case, the strong activation of one receptor system alone may be sufficient to saturate or 'exhaust' the messenger pathway.

It is evident that, when expressed in the oocyte, brain receptors to ACh, serotonin and certain other neurotransmitters are coupled in a highly nonlinear way to activate a common ionic response. The existence of a similar mechanism in neurons could have very important consequences for some brain functions, because it would make neurons highly sensitive to small changes in transmitter concentration, and the responses to one transmitter would be susceptible to 'modulation' by low concentrations of different transmitters.

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