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Receptors of the serotonin 1C subtype expressed from cloned DNA mediate the closing of K^+ membrane channels encoded by brain mRNA

(*Xenopus* oocyte/potassium channel)

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ABSTRACT The modulation of K^+ channels by serotonin (5-HT) receptors was studied by coinjecting *Xenopus* oocytes with mRNA transcribed *in vitro* from a cloned 5-HT 1C subtype (5-HT_{1C}) receptor gene, together with size-fractionated mRNA isolated from rat cerebral cortex that expresses K^+ channels. After intracellular loading with EGTA to block Ca^{2+}-dependent chloride currents, these oocytes responded to 5-HT with an inward current associated with a decrease in membrane conductance. Membrane current responses were small or absent in oocytes injected with either mRNA alone. We conclude that 5-HT_{1C} receptors are able to cause the closing of a class of K^+ channels expressed by cortex mRNA in a Ca^{2+}-independent manner. The coupling between the receptors and channels appears to be mediated by the inositol phospholipid second messenger pathway, since activation of this pathway by application of serum evoked a similar closing current.

Oocytes of *Xenopus laevis* do not normally show sensitivity to the neurotransmitter serotonin (5-HT; 5-hydroxytryptamine) (1) but develop sensitivity after injection of messenger RNA (mRNA) derived from brain (2, 3). The most prominent response in the mRNA-injected oocytes is an oscillatory Cl^- current that arises because exogenous 5-HT receptors expressed from the brain mRNA couple to an endogenous inositol phospholipid–Ca^{2+} signaling pathway in the oocyte (4–10) that leads to the opening of Ca^{2+}-dependent Cl^- channels (4, 11). In addition, loading oocytes with EGTA to block the Ca^{2+}-dependent Cl^- current reveals a smaller current that arises through the closing of a class of K^+ channels (12, 13). Pharmacological characterization of the receptors mediating the K^+-closing current indicated that they were probably of the 5-HT 1C subtype (5-HT_{1C}) (13). Furthermore, it appeared that induction of that type of response required expression of separate receptor and channel proteins, encoded by distinct mRNAs. Thus, oocytes injected with a fraction of brain mRNA that induced large Cl^- current responses to 5-HT showed little or no K^+-closing responses, whereas K^+-closing currents were seen in oocytes injected with that fraction together with a different-sized fraction of mRNA, which induced a voltage-dependent K^+ conductance (13).

We have now studied further the K^+-closing current by using the oocyte to express functional 5-HT_{1C} receptors encoded by a cloned cDNA (14). These receptors are already known to activate the inositol phospholipid–Ca^{2+} signaling system (14, 15). We show that they are, in addition, able to mediate the closing of a class of K^+ channels in a Ca^{2+}-independent manner.

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METHODS

Experiments were done on oocytes of *X. laevis* that were treated with collagenase to remove enveloping cells (1, 16). Procedures for preparation of oocytes, injection of mRNA, and electrophysiological recording were as described (13). Briefly, membrane currents were recorded from voltage-clamped oocytes in response to 5-HT applied via continuous bath superfusion of Ringer's solution at room temperature. First, oscillatory Cl^- currents were recorded at a clamp potential of −60 mV. The oocytes were then loaded intracellularly by pneumatic pressure injection of EGTA, and K^+-closing and other currents were recorded at a potential of −20 mV. Measurements of the Ca^{2+}-dependent transient outward current activated on depolarization to 0 mV (11) were also obtained to ensure that intracellular Ca^{2+} was well chelated by the EGTA.

A cloned cDNA encoding the 5-HT_{1C} receptor was kindly provided by D. Julius and R. Axel (Howard Hughes Medical Institute, Columbia University College of Physicians and Surgeons) as the plasmid $SR1_C$. This was linearized with *Not* I restriction enzyme and transcribed *in vitro* with T7 RNA polymerase to obtain capped sense 5-HT_{1C} mRNA by using a commercial mRNA capping kit (mcap; Stratagene). The conditions for transcription and measurement of mRNA concentration followed the manufacturer's instructions. Adult rat cortex mRNA was prepared as described (2, 17) and was fractionated on 10–30% (wt/wt) sucrose gradients by a modification of the procedure of Luthe (ref. 17; see ref. 18). Oocytes were each injected with 50 nl of mRNA solution prepared by mixing equal volumes of 5-HT_{1C} mRNA and fractionated brain mRNA or by mixing one of these mRNAs with an equal volume of diethyl pyrocarbonate-treated sterile distilled water. The amounts of mRNA derived from cloned cDNA encoding the 5-HT_{1C} receptor (5-HT_{1C} mRNA) and size-fractionated mRNA from rat cerebral cortex (fr. 9 mRNA) injected per oocyte were ≈0.5 and 12 ng, respectively.

RESULTS

Experiments were done on three groups of oocytes that were injected a few days before recording with either 5-HT_{1C} mRNA, fr. 9 mRNA (13), or an equal mixture of both of these mRNAs. In each oocyte, currents were first recorded at a clamp potential of −60 mV in response to bath application of 0.1 μM 5-HT. The oocyte was then loaded with EGTA, and 5-HT was reapplied at the same concentration while the oocyte was clamped at −20 mV. This potential was chosen for two reasons: K^+-closing currents are larger at less negative potentials and any residual Cl^- current not abolished by

Abbreviation: 5-HT, serotonin (5-hydroxytryptamine).

the EGTA would be outwardly directed and thus easy to identify (see ref. 13).

Representative current traces obtained in one oocyte from each group are illustrated in Fig. 1; Fig. 2 summarizes means of measurements from 6–12 oocytes from each group.

Oocytes that were injected with 5-HT_{1C} mRNA, alone or together with fr. 9 mRNA, showed large (a few microamperes) inward Cl^- currents at −60 mV, whereas oocytes injected with fr. 9 mRNA gave virtually no responses. The currents in oocytes injected with 5-HT_{1C} mRNA alone were somewhat greater than those injected with the mixture of mRNAs (respective mean currents, 3.5 and 1.9 μA), even though the amounts of 5-HT_{1C} mRNA injected in both groups were nominally identical. Reasons for the difference are not clear. It may have arisen from errors in pipetting the tiny volumes of mRNA solutions or, alternatively, the fr. 9 mRNA may have partially inhibited expression of the 5-HT_{1C} mRNA.

After loading with EGTA, responses to 5-HT were practically abolished in the oocytes injected with 5-HT_{1C} mRNA alone, and only small (mean, 4.5 nA) inward currents were seen at −20 mV. Oocytes injected with fr. 9 mRNA alone also failed to show appreciable responses. However, oocytes injected with 5-HT_{1C} plus fr. 9 mRNA gave clear inward currents at −20 mV, with a mean size of ≈70 nA. Steps in clamp potential from −20 to −30 mV were applied at intervals during recording to monitor membrane conductance. The conductance changed little, or not at all, during application of 5-HT to oocytes injected with only 5-HT_{1C} mRNA or only fr. 9 mRNA. In contrast, oocytes injected with the mixture of mRNAs showed a decrease in the current steps during application of 5-HT (Fig. 1*B*), indicating that the apparent inward current arose because of the closing of channels that are normally open at −20 mV and carry an outward current.

In addition to this current associated with a decreased conductance, some oocytes injected with both 5-HT_{1C} and fr. 9 mRNAs showed a more slowly developing increase in conductance that became apparent during the washout of

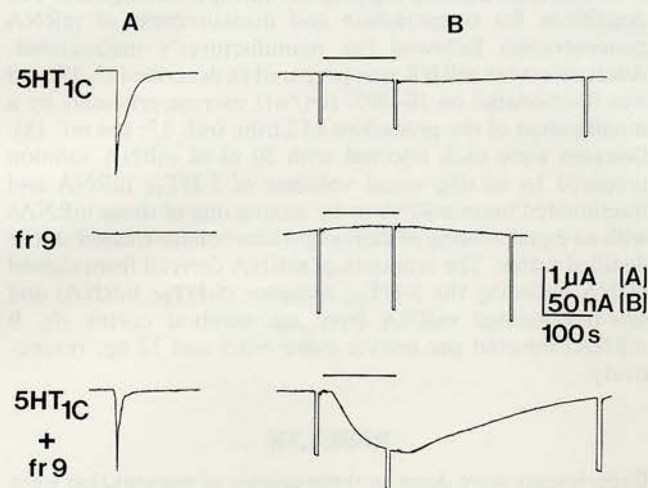

FIG. 1. Membrane currents evoked by bath application of 0.1 μM 5-HT in three oocytes injected with (from top to bottom) 5-HT_{1C} mRNA, fr. 9 mRNA from rat cerebral cortex, and a mixture of 5-HT_{1C} and fr. 9 mRNAs. Downward deflections correspond to inward membrane currents. (*A*) Records obtained at a clamp potential of −60 mV. (*B*) Records from the same oocytes as in *A* obtained at a clamp potential of −20 mV after intracellular loading with EGTA. Bars indicate the times for which 5-HT was added to the superfusate, without correction for dead time in the perfusion system. Brief steps in potential from −20 to −30 mV were given at intervals to monitor membrane conductance. Note the difference in recording gain between *A* and *B*.

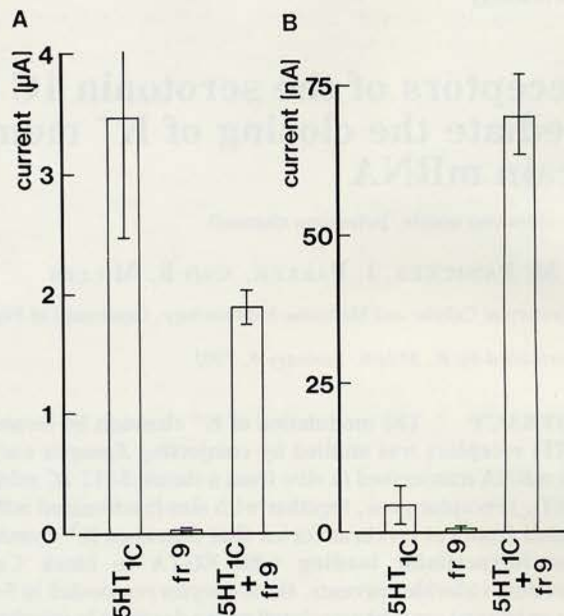

FIG. 2. Mean peak sizes of membrane currents evoked by 0.1 μM 5-HT in oocytes injected with 5-HT_{1C} mRNA, fr. 9 mRNA, or a mixture of both. (*A*) Sizes of the Cl^- current evoked at a clamp potential of −60 mV. (*B*) Sizes of the inward current recorded at −20 mV after intracellular loading with EGTA. Note the different ordinate scales in *A* and *B*. Bars show mean ± SE of measurements from 6–12 oocytes obtained from three donor frogs.

5-HT (Fig. 1*B*, lower trace). We have yet to characterize well this response, but it appears to arise through activation of a current with a reversal potential close to −20 mV, since little change in current was observed at that holding potential.

Because 5-HT_{1C} receptors activate the inositol phospholipid signaling pathway in the oocyte, we were interested to determine whether other receptors that also couple to this pathway are likewise able to induce the K^+-closing response. For this we used blood serum as an agonist, since native oocytes possess receptors to a serum factor that potently activates inositol phospholipid signaling in the oocyte (19). Rabbit serum at dilutions of 10^3 or 10^4 was bath applied to oocytes injected with fr. 9 mRNA, alone or together with 5-HT_{1C} mRNA, and currents were monitored at −20 mV after loading with EGTA. In five oocytes tested, serum evoked apparent inward membrane currents that were associated with a decrease in conductance and closely resembled the K^+-closing responses evoked by 5-HT.

DISCUSSION

In agreement with a previous report (14), we find that receptors of the 5-HT_{1C} subtype expressed from a cloned gene mediate a membrane Cl^- current like that mediated by 5-HT receptors expressed by brain mRNAs (2, 3). This current is dependent on intracellular Ca^{2+} and almost certainly arises through activation of an endogenous inositol phospholipid signaling pathway in the oocyte (7, 10). After chelating intracellular Ca^{2+} with EGTA, oocytes that were injected with only the 5-HT_{1C} mRNA showed small or no current responses. However, oocytes injected with 5-HT_{1C} mRNA together with a particular size fraction (fr. 9) of mRNA from rat brain gave inward membrane currents at a clamp potential of −20 mV. These currents were associated with a decrease in membrane conductance and arose through a closing of K^+ membrane channels (cf. ref. 13). The fr. 9 mRNA itself caused little or no expression of 5-HT receptors, since almost no sensitivity to 5-HT was seen in control or

EGTA-loaded oocytes injected with this mRNA fraction. Thus, we conclude that these Ca^{2+}-independent responses in oocytes injected with both 5-HT_{1C} and fr. 9 mRNAs arose because the exogenous 5-HT_{1C} receptors were able to modulate K^+ channels expressed from the brain mRNA. This effect was probably mediated through the inositol phospholipid signaling pathway, as similar responses were evoked by activating the pathway via native receptors to a serum factor, as well as by activation of muscarinic acetylcholine receptors (cf. ref. 13). We have yet to determine the mechanism of coupling between receptors and channels, but it is already clear that it is a Ca^{2+}-independent process.

Recently, Hoges *et al.* (20) used oocytes to demonstrate a modulatory effect of 5-HT_{1C} receptors on a K^+ current by coexpressing in the oocyte 5-HT_{1C} receptors together with mouse brain K^+ channels (MBK 1a) encoded by a cloned gene. However, those K^+ currents showed several important differences from the currents expressed by rat brain mRNA in the present and in previous experiments (13). The MBK 1a channels were of the delayed rectifier type and, unlike the maintained currents we described, were inactivated during maintained depolarization. They were also blocked by low concentrations of tetraethylammonium (IC_{50} = 0.4 mM), whereas the K^+ currents induced by fr. 9 rat brain mRNA were relatively insensitive even to high (20 mM) concentrations (13). Furthermore, the mechanisms by which the 5-HT_{1C} receptors couple to the different K^+ channels were quite different. The closing of K^+ channels that we observed did not require an elevation of intracellular Ca^{2+}, since the effect persisted after intracellular loading with EGTA. In contrast, the suppression of the MBK 1a K^+ current by 5-HT was abolished after EGTA loading and was mimicked by intracellular injection of Ca^{2+} ions (20). Thus, it seems that the 5-HT_{1C} receptor subtype is able to modulate the activity of at least two types of K^+ channels acting through different second messenger pathways.

The expression of receptors and channels from cloned genes in oocytes and other cells provides a simplified system in which to dissect the mechanisms of their interaction, with the obvious advantage that both the receptor and channel are completely defined. Nevertheless, expression from relatively crude native mRNA preparations still offers some advantages for initial exploratory studies. In particular, as was the case for 5-HT receptors, it may allow the detection of several types of functional responses arising through different channel types. Further characterization and purification of the mRNA species encoding the K^+ channels could provide a route toward their identification and cloning.

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