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Coexpression of *N*-methyl-D-aspartate and phencyclidine receptors in *Xenopus* oocytes injected with rat brain mRNA

(glutamate receptor/kainate receptor/quisqualate receptor/D-(−)-amino-5-phosphonovaleric acid/glycine potentiation)

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ABSTRACT **Recent evidence suggests that the *N*-methyl-D-aspartate (*N*-Me-D-Asp) channel is functionally and structurally associated with the phencyclidine (PCP) receptor, which mediates the psychotomimetic effects of PCP, σ opioids, and dioxalanes. To investigate the relationship between *N*-Me-D-Asp and PCP receptors on a molecular level, we injected mRNA isolated from adult rat brain into *Xenopus* oocytes. In injected oocytes *N*-Me-D-Asp application (with glycine) evoked a partially desensitizing inward current that was potentiated by glycine and blocked by D-(−)-amino-5-phosphonovaleric acid (D-APV), by Zn^{2+} and, in a voltage-dependent manner, by Mg^{2+}. These results show that the distinguishing features of rat brain *N*-Me-D-Asp channels are reproduced in this translation system. In addition, kainic acid elicited a nondesensitizing inward current at short latency, and quisqualate elicited a delayed oscillatory inward current, presumably mediated by a second-messenger system. Responses to glutamate had both short-latency and delayed components. The PCP derivative *N*-[1-(2-thienyl)cyclohexyl]piperidine (TCP) blocked the *N*-Me-D-Asp-evoked current, and its potency was comparable to its binding affinity in rat brain membranes. Onset of block required the presence of antagonist. Antagonism was stereoselective in that the active ligand dexoxadrol was a more effective blocker than its relatively inactive stereoisomer levoxadrol. Other PCP receptor ligands, (+)SKF-10,047 and MK-801, also blocked. Potencies of compounds active at *N*-Me-D-Asp and PCP receptors in oocytes were comparable to those obtained previously in electrophysiological and binding assays on neural tissues. These results indicate the coexpression of neuronal PCP and *N*-Me-D-Asp receptors in *Xenopus* oocytes.**

The excitatory neurotransmitter glutamate activates multiple receptors defined by the actions of the selective agonists kainate, quisqualate, and *N*-methyl-D-aspartate (*N*-Me-D-Asp) (1, 2). Recently, the *N*-Me-D-Asp receptor has attracted considerable attention due to its proposed roles in long-term potentiation (3, 4), developmental structuring (5), hypoxic damage (6), epileptiform seizures, and kindling (7). This receptor/channel complex is a ligand-gated cation channel with several distinct regulatory and binding sites. One of these is the site at which *N*-Me-D-Asp and the endogenous ligand glutamate act to open the channel. The competitive antagonist D-(−)-amino-5-phosphonovaleric acid (D-APV) also acts at this site. In addition, there is a positive allosteric regulatory site for glycine (8), a binding site within the channel for Mg^{2+} that blocks agonist-induced conductance in a voltage-dependent manner (9, 10), and an inhibitory site for Zn^{2+} (11, 12), which may be distinct from that for Mg^{2+}.

Recent evidence indicates that the *N*-Me-D-Asp receptor is functionally and structurally associated with the phencyclidine (PCP) receptor, which is a well-characterized site thought to mediate the behavioral actions of PCP derivatives, σ opioids, and dioxalanes (for review, see ref. 13). Drugs active at the PCP receptor noncompetitively block the actions of *N*-Me-D-Asp measured electrophysiologically (14–16) and by release assays (17, 18). Specifically, binding (19, 20) and electrophysiological (21) studies suggest that the PCP binding site is within the *N*-Me-D-Asp-activated channel. Antagonism at the *N*-Me-D-Asp receptor is stereoselective for some PCP-receptor ligands, and drug potencies in modulating *N*-Me-D-Asp effects correlate well with binding affinities for PCP receptors in brain homogenates (13, 15, 16). These findings indicate that the action of PCP on the *N*-Me-D-Asp channel is mediated by the PCP receptor. In receptor binding sites (22) antagonism between ligands of the PCP and *N*-Me-D-Asp receptors is noncompetitive. These data support the hypothesis that the PCP receptor is a binding site of the *N*-Me-D-Asp channel in a manner somewhat analogous to the benzodiazepine receptor on the γ-aminobutyric acid (GABA)-activated channel (23). Autoradiographic studies demonstrate that the two receptors are colocalized in rat forebrain and midbrain (24, 25). However, *N*-Me-D-Asp receptors occur independently of PCP receptors in the cerebellum (25).

The *Xenopus* oocyte is a self-contained expression system that is particularly suitable for the study of the relationship between receptor structure and function and for identifying the mRNA that directs receptor synthesis. This system has been shown to faithfully translate neuroreceptor (23, 26–28) and channel (29, 30) proteins. The oocyte system is capable not only of translation of the message, but also of posttranslational modifications including processing of precursor molecules (31), phosphorylation (32), and glycosylation (33), as well as assembly of subunits and insertion into the surface membrane. Expression of kainate, quisqualate (34), and, more recently, *N*-Me-D-Asp receptors (35) in *Xenopus* oocytes following injection with rat brain mRNA has been shown. In the present study we demonstrate that rat brain mRNA injected into *Xenopus* oocytes directs the synthesis of receptors for *N*-Me-D-Asp, as well as for kainate and quisqualate. The PCP receptor is coexpressed as indicated by the characteristic effects of PCP-like drugs on *N*-Me-D-Asp-induced currents.

METHODS

Isolation of mRNA. mRNA was extracted from brains minus cerebelli and brain stems of male Sprague–Dawley rats (200–250 g) using a guanidinium isothiocyanate method (36). Poly(A)$^+$RNA was isolated by oligo(dT)-cellulose chromatography (37) and stored at −70°C.

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Abbreviations: *N*-Me-D-Asp, *N*-methyl-D-aspartate; PCP, phencyclidine; TCP, *N*-[1-(2-thienyl)cyclohexyl]piperidine; D-APV, D-(−)-amino-5-phosphonovaleric acid.

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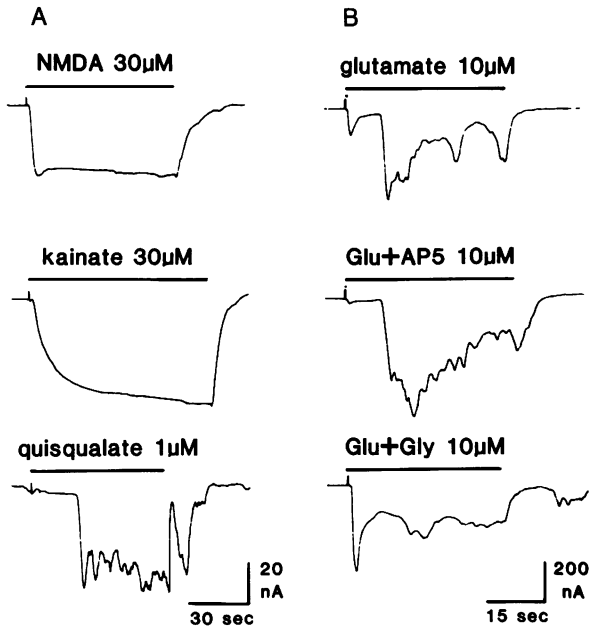

FIG. 1. Currents evoked by glutamate and its agonists. Records from oocytes clamped at −60 mV injected with mRNA 4 days previously. Horizontal bars indicate duration of application. Different cells for columns *A* and *B*. (*A*) The inward current evoked by 30 μM *N*-Me-D-Asp (NMDA) (with 10 μM glycine) was of short latency (<1 sec); it increased to an initial peak and then fell slightly to a steady-state level. The response to 30 μM kainic acid also occurred at short latency, but increased gradually to a steady-state level. The response to 1 μM quisqualate developed with a latency of 25 sec; it rose abruptly, oscillated irregularly, and outlasted drug application. The response to 10 μM glutamate was of short latency and initially like that of *N*-Me-D-Asp; then it oscillated as in responses to quisqualate. When 10 μM glutamate (Glu) and 10 μM D-APV (AP5) were applied together, the early *N*-Me-D-Asp-like component was blocked and the later quisqualate-like component was increased. When 10 μM glutamate and 10 μM glycine (Gly) were applied together, the early response was augmented, and the later response was depressed.

***Xenopus* Oocyte Injections.** Ovarian lobes were dissected from anesthetized *Xenopus laevis* and incubated (2 hr at 22°C) in Ca^{2+}-free ND96 medium (82.5 mM NaCl/2 mM KCl/1 mM $MgCl_2$/5 mM Hepes-NaOH, pH 7.5) (38) supplemented with sodium pyruvate at 2.5 mmol per liter, to which penicillin (100 units/ml), streptomycin (1 mg/ml), and collagenase (2 mg/ml) (Sigma, type 1A) were added. After transfer to Ca^{2+}-containing ND96, stage V and VI (39) oocytes were manually dissected from ovarian membranes and follicle cells and injected with mRNA (50 ng/cell). Vitelline membranes were not removed. Oocytes were maintained for 2–3 days at 16°C in Leibovitz's L-15 medium (0.7 strength) (Sigma) supplemented with 5 mM Hepes buffer, pH 7.6, penicillin (100 units/ml), and streptomycin (1 mg/ml).

Electrophysiological Recordings. Oocytes were placed in a 0.3-ml bath and perfused with Mg^{2+}-free amphibian Ringer's solution of 116 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$ buffered to pH 7.2 with 5 mM Hepes. All drugs were dissolved and applied in this medium. Cells were voltage-clamped at a holding potential of −60 mV with two beveled electrodes filled with 1 M KCl (1.5–2.5 MΩ). An agar bridge served as indifferent electrode, and all compounds were bath-applied with an access time of 1–2 sec; solutions were washed out within 2 sec as shown by visual inspection of dye application.

RESULTS

Expression of Excitatory Amino Acid Receptors. Currents evoked by application of glutamate and the selective glutamate agonists *N*-Me-D-Asp, kainate, and quisqualate to oocytes injected with mRNA from rat brain are shown in Fig. 1. At a holding potential of −60 mV, 30 μM *N*-Me-D-Asp (plus 10 μM glycine) evoked a short-latency (<1 sec) inward current that partially desensitized (within 5 sec) to a steady level that could be maintained for minutes (Fig. 1*A*, Fig. 2*A Inset*). Kainate (30 μM) produced a more slowly rising, nondesensitizing current, also of short latency, whereas quisqualate (1 μM) induced an inward current characterized by a long latency (15–30 sec), abrupt rise, and large irregular oscillations. The long latency in the case of quisqualate is suggestive of a second messenger-mediated response as previously reported (40). Glutamate (10 μM) appeared to activate an early current that resembled the response to *N*-Me-D-Asp and a later current like the response to quisqualate (Fig. 1*B Top*). The early component was largely blocked by the selective *N*-Me-D-Asp antagonist D-APV (10 μM) (Fig. 1*B Middle*), which in this system did not block kainate or quisqualate responses (data not shown). Thus, glutamate at these concentrations did not activate kainate receptors. Glycine potentiated the early glutamate response

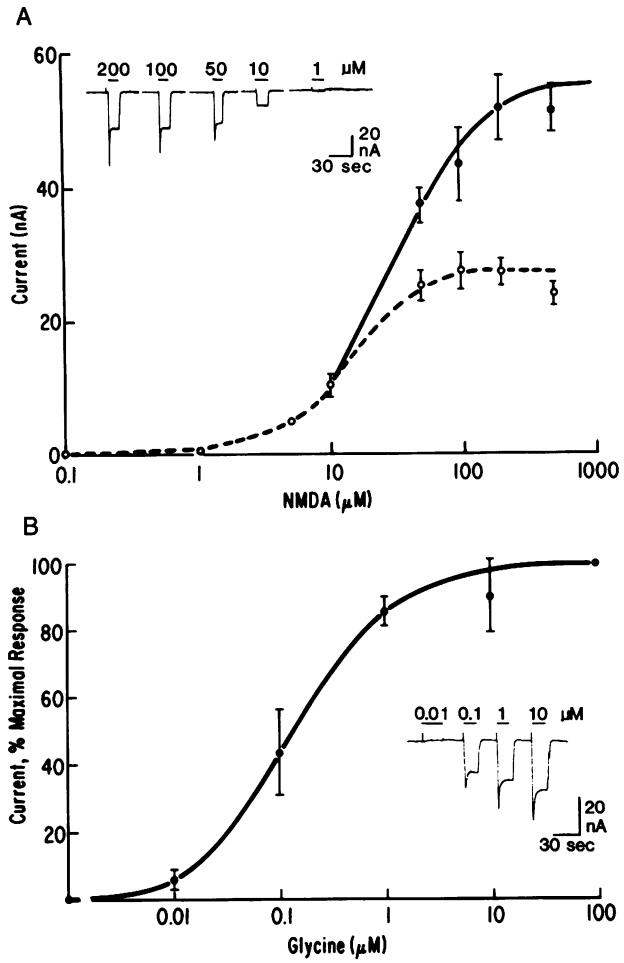

FIG. 2. Dose–response relations for *N*-Me-D-Asp (NMDA)-induced and glycine-potentiated currents. (*A*) *N*-Me-D-Asp action at 10 μM glycine. Responses (sample records *inset*) are graphed as both early peak (●) and steady-state values (○). The EC_{50} for the steady-state values was 16 ± 1.0 μM. Error bars indicate SEMs for means from three oocytes; several applications were made to each oocyte at each concentration. These oocytes had similar maximal response amplitudes. (*B*) Glycine potentiation of the response to 50 μM *N*-Me-D-Asp normalized with respect to the maximum response at 100 μM glycine. The EC_{50} was 0.19 ± 0.08 μM. Error bars and procedure as for *A*. One hundred micromolar glycine by itself had negligible effect (data not shown).

(Fig. 1*B Bottom*). When the early response to glutamate was larger, the late quisqualate-like response was reduced.

Of four mRNA preparations, all encoded *N*-Me-D-Asp receptors; in a time-course study 86% of oocytes tested (31 of 36) responded positively to *N*-Me-D-Asp 2–4 days after injection. Some oocytes tested with *N*-Me-D-Asp and kainate or quisqualate failed to express one or another receptor (6 of 17), suggesting that they are encoded by different mRNAs. Uninjected ($n = 20$) and water-injected oocytes ($n = 10$) did not respond to *N*-Me-D-Asp, kainate, or quisqualate.

Pharmacology of *N*-Me-D-Asp Responses. Responses to increasing doses of *N*-Me-D-Asp showed a half-maximal effective concentration (EC_{50}) of 16 ± 1.0 μM (mean ± SEM for three oocytes, several applications, Fig. 2*A*). At higher concentrations the initial peak was more prominent (presumably due to a greater degree of desensitization), but steady-state values were well-maintained. The delay time for bath application to the large oocytes precluded accurate determination of the maximal early response. As in neurons (8) glycine markedly potentiated the effect of *N*-Me-D-Asp (EC_{50} = 0.19 ± 0.08 μM with 50 μM *N*-Me-D-Asp) (Fig. 2*B*). There was essentially no response to 50 μM *N*-Me-D-Asp alone, and 100 μM glycine alone had negligible effect. Glycine was routinely included in *N*-Me-D-Asp solutions at a concentration of 10 μM. The *N*-Me-D-Asp-activated current was blocked by the selective *N*-Me-D-Asp antagonist D-APV (concentration for 50% inhibition, IC_{50} = 3.3 ± 0.2 μM at 50 μM *N*-Me-D-Asp) (Fig. 3*A* D-AP5), but not by its inactive L-isomer at the same concentration.

N-Me-D-Asp responses were also blocked by Mg^{2+} at potentials near the resting value (ca. −60 mV). This block was relieved by depolarization, and the relationship between *N*-Me-D-Asp-induced current and voltage exhibited a negative slope between −80 mV and −30 mV (Fig. 4). The time course of the responses was nearly constant at different voltages, even beyond the reversal potential where the responses were inverted (Fig. 4 *Inset*). When Mg^{2+} was omitted from the medium, the amplitude of the *N*-Me-D-Asp responses was a nearly linear function of voltage, and the reversal potential (−14 ± 1 mV, $n = 4$) was unchanged. The IC_{50} for Mg^{2+} was 8.3 ± 6.4 μM at a holding potential of −60 mV (Fig. 3*B*). Zn^{2+} also inhibited the *N*-Me-D-Asp responses with an IC_{50} of 9.5 ± 0.5 μM at a holding potential of −60 mV (Fig. 3*C*). Although not tested quantitatively, the dose–response curves all appeared consistent with a Hill coefficient of unity, indicating lack of cooperativity. All these properties of *N*-Me-D-Asp channels in the oocyte expression system are very similar to those of *N*-Me-D-Asp channels in rat brain neurons.

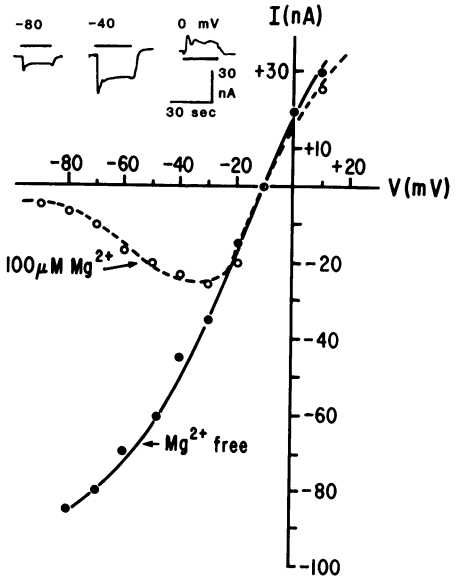

FIG. 4. Voltage dependence of Mg^{2+} block of *N*-Me-D-Asp responses. The oocyte was clamped to the indicated potentials, and the change in current was measured during application of 50 μM *N*-Me-D-Asp (plus 10 μM glycine). (*Inset*) Sample records. The response was much smaller at −90 mV with 100 μM Mg^{2+} than without Mg^{2+}. With Mg^{2+} the response increased to a maximum at −30 mV (giving the I–V relation a negative slope), whereas without Mg^{2+} the response decreased. Between −20 and +10 mV the response was similar with or without Mg^{2+}. In each case the reversal potential was −10 mV.

PCP Receptor Coexpression. PCP-receptor ligands were tested for their ability to modulate *N*-Me-D-Asp-activated currents. Application of the potent PCP derivative *N*-[1-(2-thienyl)cyclohexyl]piperidine (TCP) blocked the *N*-Me-D-Asp-evoked current in a use-dependent manner (Fig. 3*D inset*). Application of TCP together with *N*-Me-D-Asp resulted in an initial peak of inward current that at the EC_{50} for TCP was little different from the response to *N*-Me-D-Asp alone; the TCP block developed slowly. With higher concentrations the early response was reduced to some extent (Fig. 3*D inset*). The block by TCP was the same whether TCP was added before or together with agonists; TCP alone had no effect (Fig. 5*A*). The potency of TCP (IC_{50} = 0.11 ± 0.03 μM, Fig. 3*D*) in this system agrees closely with its binding affinity in rat brain homogenates under similar ionic conditions (41). As for the other dose–response relations the results appeared consistent with a Hill coefficient of one. The action

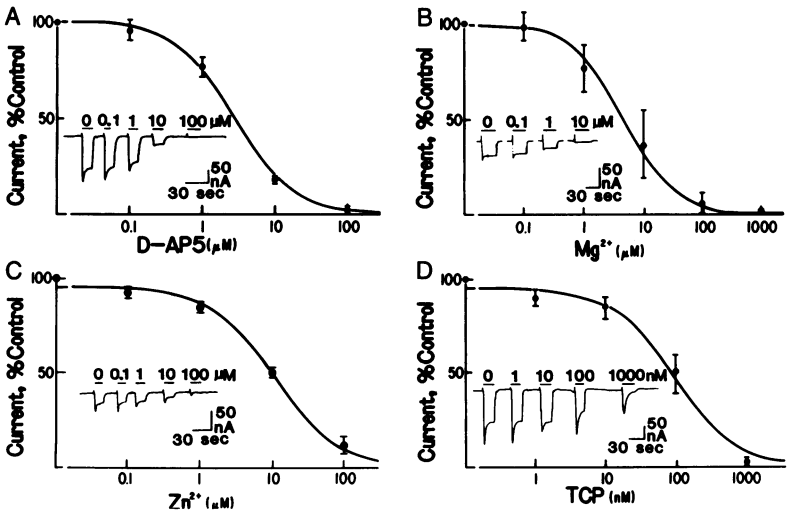

FIG. 3. Dose–response curves of antagonists of *N*-Me-D-Asp-induced currents. In each case the antagonist was added with 50 μM *N*-Me-D-Asp plus 10 μM glycine. Steady-state responses were normalized with respect to the control responses. Sample records are inset in each graph. (*A–D*) Action of D-APV (D-AP5), Mg^{2+}, Zn^{2+}, and TCP. Procedure as for Fig. 2 except the block by high concentrations of TCP was slowly reversible, and these concentrations were applied last.

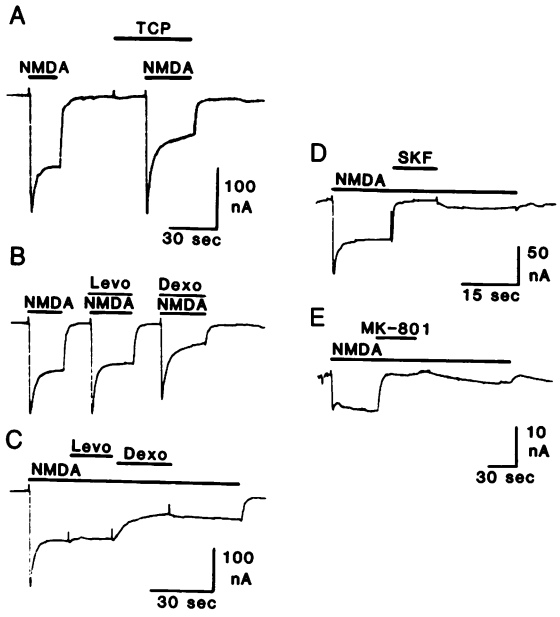

FIG. 5. Use dependence of TCP block and other pharmacology. Time of drug applications indicated by bars. (*A*) Application of 50 μM *N*-Me-D-Asp (NMDA) (plus 10 μM glycine) produced a typical partially densensitizing response. After washing and application of 0.1 μM TCP, 50 μM *N*-Me-D-Asp (plus 10 μM glycine) with TCP produced a response that was slowly blocked. The initial peak was little different from that evoked by *N*-Me-D-Asp alone. (*B*) Stereoselectivity of the PCP receptor. Fifty micromolar *N*-Me-D-Asp (plus 10 μM glycine) was applied alone, with 1 μM levoxadrol, and with 1 μM dexoxadrol. The initial response was only slightly reduced by dexoxadrol. The steady-state response was depressed ≈50% by dexoxadrol but <10% by levoxadrol. (*C*) Without washing between applications the same block of the steady-state currents was observed; the block by dexoxadrol developed slowly as in *B*. (*D*) Block by 10 μM (+)SKF-10,047 added during application of 50 μM *N*-Me-D-Asp (plus 10 μM glycine). Recovery was slow even in the presence of agonist. (*E*) Block by 1 μM MK-801 added during application of 50 μM *N*-Me-D-Asp (plus 10 μM glycine). Recovery was slow.

of TCP differed markedly from that of Mg^{2+} and D-APV, which blocked the early *N*-Me-D-Asp response when applied simultaneously with *N*-Me-D-Asp. D-APV as a competitive blocker would prevent *N*-Me-D-Asp from reaching its binding site. Mg^{2+} as a channel blocker would reach its binding site in the channel more rapidly than TCP. The rate of recovery from the TCP block was very slow, even in the presence of *N*-Me-D-Asp.

The PCP receptor was stereoselective in that *N*-Me-D-Asp responses were reduced by the active ligand dexoxadrol (1 μM) but little affected by its relatively inactive stereoisomer levoxadrol at the same concentration (Fig. 5 *B* and *C*). The prototypic σ opioid, (+)SKF-10,047 (10 μM) also blocked *N*-Me-D-Asp-induced currents (Fig. 5*D*). In addition, MK-801 (1 μM), a new and extremely potent PCP receptor ligand (42), inhibited *N*-Me-D-Asp-evoked currents (Fig. 5*E*) without effect on quisqualate- or kainate-evoked currents. Recovery from MK-801 was very slow. Table 1 summarizes the potencies of these ligands determined in the oocyte. The rank order of potency of PCP receptor ligands in inhibiting the *N*-Me-D-Asp-evoked response was apparently MK-801 > TCP, (+)SKF-10,047 > dexoxadrol > levoxadrol. These data indicate that many of the pharmacological properties of the *N*-Me-D-Asp and PCP receptors are reproduced in the oocyte-expression system. Most important, in every oocyte tested, PCP-receptor ligands reduced or blocked *N*-Me-D-Asp-induced current, demonstrating coexpression of their receptors (25 of 25, including oocytes injected with mRNA from specific brain regions or size-fractionated).

DISCUSSION

This study demonstrates the expression of *N*-Me-D-Asp, kainate, and quisqualate receptors in *Xenopus* oocytes injected with adult rat brain mRNA. The *N*-Me-D-Asp channel expressed in the oocyte was potentiated by glycine and blocked by Mg^{2+}, Zn^{2+}, and PCP-receptor ligands. This result demonstrates coexpression and functional association of the PCP receptor with the *N*-Me-D-Asp channel. Our findings provide direct evidence in support of the hypothesis that the PCP receptor is a site associated with the *N*-Me-D-Asp-activated channel. The agonist dependence of onset of block (Figs. 3*D* and 5*A*) is consistent with location of the PCP receptor within the channel, where it is accessible only when the channel is open (21). Our quisqualate-activated currents were of long latency; this finding is suggestive of mediation by a second-messenger system, as was previously reported (40). The suggestion that *N*-Me-D-Asp, kainate, and quisqualate receptors operate the same channels (43, 44) is contradicted by our observation that block of *N*-Me-D-Asp-induced currents by MK-801 does not block responses to the other agonists. Moreover, macromolecular complex (43, 44) is not directly addressed by our study, although limited data suggest independent expression. The *N*-Me-D-Asp response appeared to depress the long-latency quisqualate response so that their amplitudes were inversely related. The mechanism involved in this interaction is unclear; the delay in the quisqualate response makes it unlikely that the two currents use the same channels.

The pharmacological properties of *N*-Me-D-Asp receptors expressed in *Xenopus* oocytes following injection of rat brain mRNA are similar to those of *N*-Me-D-Asp receptors in spinal, striatal, cortical, and hippocampal neurons (8–16). The receptors in all three systems are activated by *N*-Me-D-Asp and glutamate, potentiated by glycine, and blocked by the selective competitive antagonist D-APV and by Mg^{2+} and Zn^{2+}. Block by Mg^{2+} is marked at negative potentials and relieved by depolarization. The potencies of glycine, Mg^{2+}, and Zn^{2+} are comparable in oocytes and neurons.

The regulation of *N*-Me-D-Asp-activated currents by PCP-receptor ligands is similar in oocytes and in neurons and occurs at similar concentrations. Moreover, the potencies observed in the oocyte are consistent with binding studies of isolated membranes (41, 42). Block by TCP did not develop in the absence of agonist, a result which suggests that TCP binds within the channel. Others reported that for other PCP-receptor ligands acting on neurons, agonist is required to relieve as well as produce block (20). In the oocyte system,

Table 1. Potency of *N*-Me-D-Asp and PCP receptor ligands expressed in *Xenopus* oocytes

Compound	Probable action	EC_{50} or IC_{50}, μM
N-Me-D-Asp	Agonist	16.0 ± 1.0 (3)
Glycine	Potentiator	0.19 ± 0.08 (3)
D-APV	Competitive inhibitor	3.3 ± 0.2 (4)
Mg^{2+}	Channel blocker	8.3 ± 6.4 (3)
Zn^{2+}	Noncompetitive inhibitor	9.5 ± 0.5 (3)
TCP	Channel blocker	0.1 ± 0.03 (4)
		% inhibition at dose, μM
Dexoxadrol	Channel blocker	54 ± 5% 1 (5)
Levoxadrol	Channel blocker	9 ± 4% 1 (5)
MK-801	Channel blocker	91 ± 5% 0.1 (5)
(+)SKF-10,047	Channel blocker	36 ± 16% 0.1 (5)

Values are means ± SEM of mean responses for single oocytes. Numbers of oocytes are indicated in parentheses.

the action of agonists on recovery from block by PCP receptor ligands requires further study.

If the PCP receptor is, indeed, a site in the *N*-Me-D-Asp-activated channel, one would expect that, as we observed, *N*-Me-D-Asp and PCP receptors would always be coexpressed. However, autoradiographic studies indicate that in the cerebellum *N*-Me-D-Asp binding sites occur at much greater density than PCP binding sites (25). The oocyte expression system should prove useful for physiological characterization of receptor subtypes encoded by mRNAs from different regions and ultimately for identification and cloning of these mRNAs.

Note Added in Proof. Recent work shows that in oocytes PCP reduces *N*-Me-D-Asp currents (IC_{50} = 27 ± 7 nM, n = 3) in a use-dependent manner. Use dependence of recovery is easily demonstrated, but some recovery occurs without applied agonist. Recovery in the presence of agonist is greatly speeded at inside positive voltages, as was previously observed in neurons (cf. ref. 21).

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1. Mayer, M. L. & Westbrook, G. L. (1987) *Prog. Neurobiol.* **28,** 197–276.
2. Foster, A. C. & Fagg, G. E. (1984) *Brain Res. Rev.* **7,** 103–164.
3. Wigstrom, H. & Gustaffson, B. (1984) *Neurosci. Lett.* **44,** 327–332.
4. Harris, E. W., Ganong, A. H. & Cotman, C. W. (1984) *Brain Res.* **323,** 132–137.
5. Rauschecker, J. P. & Hahn, S. (1987) *Nature (London)* **326,** 183–185.
6. Simon, R. P., Swan, J. H., Griffiths, T. & Meldrum, B. S. (1984) *Science* **226,** 850–852.
7. Dingledine, R., Hynes, M. A. & King, G. L. (1986) *J. Physiol. (London)* **380,** 175–189.
8. Johnson, J. W. & Ascher, P. (1987) *Nature (London)* **325,** 529–531.
9. Nowak, L., Bregestovski, P., Ascher, P., Herbert, A. & Prochiantz, A. (1984) *Nature (London)* **307,** 462–465.
10. Mayer, M. L., Westbrook, G. L. & Guthrie, P. B. (1984) *Nature (London)* **309,** 261–263.
11. Peters, S., Koh, J. & Choi, D. W. (1987) *Science* **236,** 589–593.
12. Westbrook, G. L. & Mayer, M. L. (1987) *Nature (London)* **328,** 640–643.
13. Zukin, R. S. & Zukin, S. R. (1988) in *The Opiate Receptors*, ed. Pasternak, G. (Humana, New Jersey), in press.
14. Honey, C. R., Miljkovic, Z. & MacDonald, J. F. (1985) *Neurosci. Lett.* **61,** 135–139.
15. Anis, N. A., Berry, S. C., Burton, N. R. & Lodge, D. (1983) *Br. J. Pharmacol.* **79,** 565–575.
16. Berry, S. C., Dawkins, S. L. & Lodge, D. (1984) *Br. J. Pharmacol.* **83,** 179–185.
17. Snell, L. D. & Johnson, K. M. (1985) *J. Pharmacol. Exp. Ther.* **235,** 50–57.
18. Snell, L. D. & Johnson, K. M. (1986) *J. Pharmacol. Exp. Ther.* **238,** 938–946.
19. Reynolds, I. J., Murphy, S. N. & Miller, R. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7744–7748.
20. Kloog, Y., Haring, R. & Sokolovsky, M. (1988) *Biochemistry*, in press.
21. MacDonald, J. F., Miljkovic, Z. & Pennefather, P. (1987) *J. Neurophysiol.* **58,** 251–266.
22. Javitt, D. C., Jotkowitz, A., Sircar, R. & Zukin, S. R. (1987) *Neurosci. Lett.* **78,** 193–198.
23. Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandran, J., Reale, V., Glencorse, T. A., Seeburg, P. H. & Barnard, E. A. (1987) *Nature (London)* **328,** 221–227.
24. Maragos, W. F., Chu, D. C. M., Greenamyre, T., Penney, J. B. & Young, A. B. (1986) *Eur. J. Pharmacol.* **123,** 173–174.
25. Jarvis, M. F., Murphy, D. E. & Williams, M. (1987) *Eur. J. Pharmacol.* **141,** 149–152.
26. Sumikawa, K., Houghton, M., Emtage, J. S., Richards, B. M. & Barnard, E. A. (1981) *Nature (London)* **292,** 862–864.
27. Sakai, Y., Kimura, H. & Okamoto, K. (1986) *Brain Res.* **362,** 199–203.
28. Parker, I., Gundersen, C. B. & Miledi, R. (1985) *Proc. R. Soc. London Ser. B* **226,** 263–269.
29. Gundersen, C. B., Miledi, R. & Parker, I. (1984) *J. Physiol. (London)* **353,** 231–248.
30. Barnard, E. A., Miledi, R. & Sumikawa, K. (1982) *Proc. R. Soc. London Ser. B* **215,** 241–246.
31. Ghysdael, J., Hubert, E., Travnicek, M., Bolognesi, D. P., Burny, A., Cleuter, Y., Huez, G., Kettmann, R., Marbaix, G., Portetelle, D. & Chantrenne, H. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3230–3234.
32. Gedamo, L., Dixon, G. H. & Gurdon, J. B. (1978) *Exp. Cell Res.* **117,** 325–335.
33. Lund, T., Bravo, R., Johansen, H. R., Zeuthen, J. & Vuust, J. (1986) *FEBS Lett.* **208,** 369–372.
34. Houamed, K. M., Bilbe, G., Smart, T. G., Consanti, A., Brown, D. A., Barnard, E. A. & Richards, B. M. (1984) *Nature (London)* **310,** 318–321.
35. Verdoorn, T. A., Kleckner, N. W. & Dingledine, R. (1987) *Science* **238,** 1114–1116.
36. Ullrich, A., Shine, J., Chirgwin, J., Pictet, R., Tischer, E., Rutter, W. J. & Goodman, H. M. (1977) *Science* **196,** 1313–1319.
37. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
38. Dascal, N., Snutch, T. P., Lubbert, H., Davidson, N. & Lester, H. A. (1986) *Science* **231,** 1147–1150.
39. Dumont, J. N. (1972) *J. Morphol.* **136,** 153–180.
40. Sugiyama, H., Isao, I. & Hirono, C. (1987) *Nature (London)* **325,** 531–533.
41. Zukin, S. R. & Zukin, R. S. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5372–5376.
42. Sircar, R., Rappaport, M., Nichtenhauser, R. & Zukin, S. R. (1987) *Brain Res.* **435,** 235–240.
43. Jahr, C. E. & Stevens, C. F. (1987) *Nature (London)* **325,** 522–525.
44. Cull-Candy, S. G. & Usowicz, M. M. (1987) *Nature (London)* **325,** 525–528.