amine site remains to be documented.) Moreover, this NMDA-R1 subunit has clear sequence homology to the non-NMDA receptor GluR1 series over much of its sequence and this homology is very strong in localized regions in or near some putative transmembrane domains. Yet, in expression it does not respond to non-NMDA agonists but strongly to NMDA.

By contrast, in the case of the Michaelis GBP, some important questions are indeed left open. The starting protein preparation was purified using only a column of L-glutamate immobilized on an NH_2-reactive matrix, although the free NH_2 group of L-glutamate is essential for its NMDA receptor activity; this is unexplained. There are unsupported bald statements ('data not shown') on the ligand-binding properties of the purified protein, which are crucial to its authentication as an NMDA receptor. No specific binding activity for any ligand is stated; the enrichment mentioned, if taken with the starting homogenate data from the reference cited[10], would give only 200 pmoles of [^{3}H]glutamate binding sites per mg purified protein, or less than 2% purity. The protein, when incorporated in liposomes, gave glutamate-activated ion flux[11], but it contains[3] six clearly demonstrable polypeptides of 70 kDa down to 31 kDa (in line with its low specific activity) and only one of these (70 kDa) was used to proceed to the cloning. Antibody expression screening in λZap was used to isolate the GBP clone. Again the immobilized-glutamate column was used to isolate the subunit/β-galactosidase fusion protein (which is now ~60 kDa). It bound L-glutamate (a K_d of 0.26 μM was noted) but no test for channel activity nor specific agonist or antagonist binding nor any other property of the NMDA receptor is reported for it[3]. The identification evidence is circular: the product is recognized by the same gel matrix and the same antibody that were applied to recognize the initial protein. That antibody was raised against a protein that bound glutamate but not NMDA[10].

This GBP protein has no homology to any known glutamate receptor subunit or kainate-binding proteins. It does not have the deduced topology of other glutamate receptor subunits. However, it has some homology to an enzyme (a transpeptidase) which recognizes the γ-glutamyl group[3]. Glutamate binding is a property of a number of non-receptor proteins; it occurs not only in several enzymes, but also, for example, on brain membranes, which contain many sites of high-affinity [^{3}H]L-glutamate binding that are on complexes of cytochrome b (T. Volkova and E. Grishin, unpublished) or on other, unidentified proteins. When the purification of the antigenic protein used for the cloning was repeated by others, high levels of a protein that binds glutamate but not NMDA, and not attributable to a receptor, were found[12].

The question to be asked should not be, therefore, which of the two proteins[1,3] is from an NMDA receptor. The conclusion can be drawn that the NMDA-R1 subunit is indeed from such a receptor, at an immensely greater level of confidence than for GBP. Nevertheless, the original reason given for stating[7] that one of the two *must* be wrong is in error, as argued above. A great deal more evidence is now needed on the purified GBP of the Michaelis group, as well as on the properties and functionality of their recombinant polypeptide, before this question can be settled.

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LETTERS

P-450 might provide signal for state of Ca^{2+} stores

Ca^{2+} influx following receptor activation

A recent *TiPS* article from Meldolesi *et al.* (August 1991)[1] on mechanisms facilitating Ca^{2+} influx following receptor activation analysed a derivation of Putney's capacitative model[2], where Ca^{2+} influx may be secondary to the emptying of the intracellular Ca^{2+} stores induced by the agonists; 'an undefined signal generated at the empty intracellular rapidly exchanging Ca^{2+} stores' being responsible for the opening of plasma membrane Ca^{2+} channels. We have proposed recently that such an 'undefined signal' may be generated by a microsomal cytochrome P-450. This proposal is based on the effects of several cytochrome P-450 inhibitors, which prevent the acceleration of Ca^{2+} (or Mn^{2+}) entry through the plasma membrane that is induced by emptying the intracellular Ca^{2+} stores in thymocytes[3], neutrophils[4] and platelets[5]. These inhibitors have no effect on agonist-induced release of Ca^{2+} from the intracellular Ca^{2+} stores in these cells[3–5].

The inhibitory effects of SK&F-96365 and SC38249 (Ref. 1) may have the same basis. Both are N_1-substituted non-polar imidazole derivatives and many similar compounds are known to inhibit cytochrome P-450 (Ref. 6). We find that SK&F96365 inhibits cytochrome

P-450-mediated *O*-dealkylase activity in liver microsomes[5] [representative IC_{50} value 5–10 μM (C. Villalobos, unpublished), cf. 8–12 μM for inhibition of agonist-induced Ca^{2+} entry in neutrophils and platelets and 3–25 μM for inhibition of voltage-gated Ca^{2+} entry in pituitary GH_3 cells and smooth muscle cells[7]].

In our model, cytochrome P-450, sited at the stores, is able to sense $[Ca^{2+}]$ within the stores by a mechanism sensitive to calmodulin antagonists[3]. Decrease of $[Ca^{2+}]$ within the stores would disinhibit cytochrome P-450, causing a direct or indirect (via a metabolite travelling from the stores to the plasma membrane) opening of plasma membrane Ca^{2+} channels. Receptor-activated Ca^{2+} entry in human neutrophils[4] and platelets[5] could be explained by this mechanism.

Alternatively, cytochrome P-450 inhibitors may interact directly with the channels. Imidazole antimycotics and other cytochrome P-450 inhibitors also block Ca^{2+} entry through voltage-gated channels (C. Villalobos, unpublished), as do SK&F96365 (Ref. 7) and SC38249 (Ref. 8). These results may suggest structural similarities between the Ca^{2+} entry pathways activated by agonists and by depolarization. These similarities may condition either direct interaction with the same inhibitors or regulation by similar redox mechanisms.

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High-dose tetanus and the role of reduced inhibitory tone

Mechanism of tetanus toxin in neuronal cell death

Bagetta *et al.* have written a clear and concise account of the actions of tetanus toxin on the mammalian CNS (August *TiPS*, pp. 285–289)[1]. However, I believe that their discussion of the neuronal degeneration that they find seven days after injecting the toxin into the hippocampus could be misleading because of the very high doses used, these being two orders of magnitude greater than those used by other groups working on the medium- to long-term effects of intracranial tetanus toxin.

The authors conclude that the neuronal loss following intrahippocampal tetanus toxin shows that 'reduced inhibitory tone' can cause neuronal death. To get these effects they use doses of 1000 mouse minimum lethal doses (MLD). Others studying the chronic effects of the toxin typically use 6–20 MLD[2–5]. At these lower doses, neurons (and the rats!) survive, as has been shown by Bagetta *et al.*[6] and by many others[2,7,8]. These same low doses selectively disrupt neuronal inhibition in these animals, which most likely explains why they become chronically epileptic. Thus 10–14 days after injecting 6–12 MLD of tetanus toxin, the *ex vivo* release of GABA from intact hippocampal slices decreased to one third of control[9], and inhibitory postsynaptic potentials (IPSPs) recorded from individual neurons within the epileptic focus decreased to one tenth[10]. Given that these rats normally retain a full complement of hippocampal neurons, a substantial loss of inhibition is clearly not sufficient for cell death[8,11].

The very high doses of toxin used by Bagetta *et al.* raise doubts about its selectivity for inhibition. Similar high doses applied acutely to slices do block IPSPs more readily than excitatory postsynaptic potentials (EPSPs)[12] (Fig. 3 of Ref. 1). However, the effects of the toxin on synaptic transmission are both time and dose dependent[13,14]. Even if the high-dose toxin selectively blocks IPSPs initially, it will go on to block fast EPSPs and other cellular functions during the following hours to days. Indeed, one of the remarkable features of the toxin is the variety of transmitter systems in which it can block release[15]. As far as I am aware, the relevant measurements seven days after intrahippocampal injection of 1000 MLD of tetanus toxin simply have not been made. We do know that inhibition is selectively impaired 10–14 days after injection of 6–12 MLD[9,10], presumably because these doses are so low that the toxin is cleared from the brain before it can block EPSPs. Far from causing a 'loss of inhibitory tone', doses of 1000 MLD of tetanus toxin will cause a complex sequence of cellular changes that will depend on time, distance from the injection site and the relative sensitivities of different types of neuron and terminal to the toxin.

I agree that intracranial injections of tetanus toxin can contribute to our understanding of neuropathology. Low doses (<12 MLD) of intrahippocampal tetanus toxin can cause histopathology that is much more subtle than that found with the high (1000 MLD) doses[1]. Both Mellanby's group and my own have found sporadic cases of neuronal death over the years[16] in our respective studies of the chronic epileptic foci established by the toxin. In a recent quantitative histological study of unilateral injections of tetanus toxin[11] we found three cases of neuronal loss out of 33 rats; the remaining 30 retained normal numbers of hippocampal neurons. Where lesions occurred, they were in CA1 contralateral to the injection, in contrast to the ipsilateral lesions found with high-dose toxin[1]. Independent epileptic foci