Chemical modification of spinal cord membranes reveals [3H]strychnine binding sites that are not located on the 48 kDa subunit of the synaptic glycine receptor

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High-affinity binding sites for the convulsant alkaloid strychnine appear to reside on a 48 kDa subunit of receptors for the inhibitory neurotransmitter glycine in the mammalian spinal cord [1, 2]. During a series of experiments to investigate the role of different amino acid residues in the recognition of strychnine at the glycine receptor, we found that modification of arginine residues with 2,3-butanedione (BD; 80 mM for 40 min in phosphate-buffered saline (100 mM NaCl, 50 mM-Na₂HPO₄/NaH₂PO₄, pH 7.4, 20°C)) increased [3H]strychnine binding to mouse spinal cord membranes [3, 4]. This BD-induced binding (with the 3H-labelled ligand at 6 nM) was specific in that it could be inhibited by unlabelled strychnine (10⁻⁴ M). However, BD treatment was found to abolish the ability of glycine (10⁻⁴ M) to inhibit [3H]strychnine binding. Further experiments have now shown that BD treatment reveals additional [3H]strychnine binding sites in these membranes, sites which do not appear to reside on the previously characterized [5] synaptic glycine receptor complex.

We have examined the distribution of the BD-induced increase in [3H]strychnine binding by treating membranes prepared from different regions of the mouse central nervous system. A significant correlation (P < 0.05; Spearman rank correlation coefficient) was found between the amount of specific, strychnine-displaceable, [3H]strychnine binding in control untreated membranes and the magnitude of the increase in binding of this ligand after BD treatment; both parameters being highest, for example, in the spinal cord and lowest in the cerebellum. The distribution of the strychnine binding sites revealed by BD treatment, therefore, appears to parallel the distribution of the high-affinity binding sites for this ligand in untreated membranes. In contrast, BD-induced [3H]strychnine binding to spinal cord membranes prepared from the mutant mouse spastic was not significantly different (P > 0.05; unpaired t-test) from that to spinal cord membranes prepared from unaffected littermate control mice. This occurred despite a reduction in high-affinity [3H]strychnine binding to untreated membranes to less than 20% of control in the mutant.

Further evidence that the BD-induced strychnine-binding sites arise from structures distinct from those identified by high-affinity [3H]strychnine binding has come from experiments on synaptic glycine receptors solubilized in 1.5% (w/v) Chaps from mouse spinal membranes and affinity purified on a 2-aminopimelic acid-agarose column [6]. When treated with BD, these purified receptors do not show an increase in [3H]strychnine binding. As found in whole membrane preparations, however, this BD-treatment of purified receptors abolishes the ability of glycine, but not unlabelled strychnine, to inhibit [3H]strychnine binding.

Strychnine can be used as a photoaffinity label for the glycine receptor [1]. However, the strychnine-binding sites revealed by BD-treatment of spinal-cord membranes failed to incorporate [3H]strychnine irreversibly upon illumination with ultraviolet light (nominal peak intensity at 254 nm of 300 μW/cm², for 30 min, 4°C). Indeed, the amount of radioactivity incorporated into these BD-treated membranes was similar to that incorporated into untreated membranes. Solubilization of the photolabelled membranes (in 10% (w/v) SDS) followed by SDS/PAGE and fluorography showed that the [3H]strychnine was incorporated into a 48 kDa peptide in both BD-treated and untreated membranes.

Preliminary kinetic studies suggest that the strychnine-binding sites revealed by BD-treatment of spinal cord membranes are of a low affinity, with a Kᵣ of approximately 1 μM. Although these binding sites appear to coexist in normal spinal cord membranes with the high affinity (Kᵣ approximately 6 nM) strychnine-binding sites previously identified [7, 8], the present evidence suggests that they are not located on the same multi-subunit glycine receptor. Further characterization of these low-affinity strychnine binding sites may indicate their physiological role.

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Abbreviation used: BD, butanedione.