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**EFFECTS OF BILIRUBIN ON GLUTAMATE UPTAKE BY SYNAPTOMES, NMDA RECEPTOR BINDING IN SYNAPTIC MEMBRANES, AND GLUTAMATE RECEPTORS EXPRESSED IN OOCYTES .**

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Altered neurotransmission produced by bilirubin (B) binding to nerve terminals may contribute to B induced neurotoxicity. We studied the effects of B on glutamate (Glu) uptake by synaptosomes and NMDA receptor binding in 6 week old Sprague Dawley rats and 7 day pups (age of greatest vulnerability to cerebellar hypoplasia). Effects of B on rat brain Glu receptors expressed in *Xenopus* oocytes was studied by measuring ion channel activation by B, Glu, and Glu sub-group activators.

**Glutamate Uptake:** B (B/albumin molar ratio 1:10) inhibited 3H-Glu uptake in dihydrokainate sensitive striatal synaptosomes (P2 fraction); with an IC<sub>50</sub> (B concentration producing 50% inhibition) of 24  $\mu$ M. Cerebellar fractions (L-alpha-aminodipate sensitive) were more sensitive to B; IC<sub>50</sub>=14  $\mu$ M in adult rats and only 7.7  $\mu$ M in 7 day pups.

**NMDA Receptor Binding:** Disclosure of NMDA sensitive Glu receptors in crude synaptic membranes was enhanced by treating membranes with triton X-100. Following incubation with 3H-Glu, about 85% of bound Glu was displaced by 1.0 mM cold glutamate (defining specific binding), or by 100  $\mu$ M NMDA. Double reciprocal plots of initial binding velocity using varying concentrations of B and 3H-Glu indicated that both Km and Vmax were affected. 50% displacement of maximum equilibrated Glu binding occurred at the following B concentrations:

	7 days	6 weeks
Cerebellum	2.5 $\mu$ M	5.1 $\mu$ M
Striatum	5.2 $\mu$ M	16.5 $\mu$ M

**Glutamate Receptor-Mediated Ion Currents:** Glu receptors were expressed in *Xenopus* oocytes microinjected with mRNA from rat brains. Glu and sub-group activators NMDA, AMPA, kainate, and quisqualate each induced characteristic ion currents. B (0.2-20  $\mu$ M) alone had no effect on ion currents, and B neither inhibited nor augmented ion current response to Glu or sub-group activators.

**Conclusions:** Both Glu uptake and NMDA receptor binding were most sensitive to B in the developing cerebellum, a target organ for B toxicity in the rat. However, the anticipated interaction of B with Glu receptor function was not confirmed in the oocyte model used in these experiments