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Title : Pentobarbital Anesthesia Increases GABA Receptor Density in Rat Cerebral Cortex

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Introduction. Pentobarbital depresses neuronal excitability by activating GABA-receptor coupled Cl^- conductance and potentiating GABA-induced changes in postsynaptic membrane.[1] Pentobarbital also inhibits potassium-stimulated release of GABA from cerebral cortical slices[2] and calcium-dependent efflux of GABA from brain synaptosomes.[3] We studied GABA receptor binding characteristics using ^{3}H-muscimol, a GABA receptor agonist, and synaptosomes prepared from cerebral cortex of rats anesthetized with pentobarbital.

Methods. Male Sprague-Dawley rats, 300-350 g, were used. Pentobarbital sodium, 60 mg/kg, was injected iv in the morning hours. Body temperature was monitored and kept at 38-39° C with a heating pad. Rats were decapitated at 30 min following the injection, at the time of return of righting reflex (1.5 - 2 hrs), and at 24 hrs. Control, unanesthetized rats were killed at approximately the same time.

Cerebral cortex was frozen for at least 24 hrs, homogenized in 50 vol of 50 mM Tris-citrate buffer, pH 7.1. After centrifugation at 46,000 x g for 10 min, the pellet was washed once with the buffer, and incubated with Triton X-100 (0.05 percent) at 37° C for 20 min. The washing was repeated. The pellet was then resuspended in 25 vol of buffer containing 1 - 1.5 mg protein/ml, used for GABA receptor binding assay. Specific binding was measured with ^{3}H-muscimol (7.3 Ci/mmol) as the ligand in concentrations ranging from 1.4 to 7 nM, in the presence or absence of 1 mM GABA. The assay was carried out in duplicate, the mixture incubated at 23° C for 20 min, stopped by filtering on Whatman GF/C glass fiber discs and washing with ice-cold buffer. Discs were dried, placed in minivials and 4 ml of Aquasol added for scintillation counting. Specifically bound muscimol and bound/free ratios with five muscimol concentrations were calculated. Scatchard analysis gave GABA receptor density (Bmax) in fmol/mg protein and receptor affinity (K_D) for the ligand in nM.

Specific binding of ^{3}H-muscimol to synaptosomes in the presence of pentobarbital, 25-225 μM, was also measured using cerebral cortex of control rats.

Student t test for unpaired data was used to compare results obtained from control and pentobarbital-anesthetized animals.

Results. Specific binding of ^{3}H-muscimol to GABA receptors at a concentration of 7 nM accounted for 30-40 percent of the ligand. Pentobarbital, 25-225 μM, added to the incubation mixture containing synaptosomes prepared from control rats, did not alter specific binding. Bmax of cortical tissue of control rats was 1910 ± 66 (mean ± SE) fmol/mg protein and K_D, 4.78 ± 0.31 nM (n = 15). At 30 min following iv injection of pentobarbital, Bmax increased to 2310 ± 146 fmol/mg protein (n = 11, $P < 0.02$ compared to control). K_D did not change significantly. At the time of return of righting reflex and 24 hrs later, Bmax were 1880 ± 231 (n = 4) and 1980 ± 162 (n = 7) fmol/mg protein, respectively; K_D remained the same.

Discussion. Pentobarbital, in clinically relevant concentrations, did not change GABA receptor affinity to ^{3}H-muscimol in vitro. This is in agreement with published reports that barbiturates do not bind with GABA receptors per se.[4] The increase in GABA receptor density in the cerebral cortex during pentobarbital anesthesia (21 percent) was transient. This change could be an adaptive mechanism in response to decreased release and availability of the transmitter,[2,3] although the postsynaptic inhibitory action of GABA is enhanced.[1] The transient increase in cortical GABA receptor density could also explain the phenomenon of acute tolerance to barbiturates, in that a greater number of GABA receptors is present with reduced availability of the transmitter. Chronic treatment of rats with phenobarbital, 30 mg/kg, ip for 30 days, has been found to decrease GABA receptor density in the striatum.[5] Nordberg et al reported recently that in rats, upon abrupt withdrawal of chronic administration of barbital (3.33 mg/ml in drinking water, 42 weeks), the peak frequency of convulsion on day 3 was associated with significant increases in muscarinic receptor density in the striatum and cerebellum-lower brain stem.[6] It seems that anesthetic action on characteristics of brain receptors for various neurotransmitters warrants closer examination.

References

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