Anti-serotonergic effects of urethane and chloral hydrate may not be mediated by a blockade of 5-HT$_2$ receptors

Short Communication

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Summary. The general anesthetics urethane and chloral hydrate have profound anti-serotonergic effects both in the rat cortex in vivo and the rat aortic ring in vitro. The suggestion that these effects may be due to an action on 5-HT$_2$ receptors was tested using ex vivo and in vitro $[^3]$H]ketanserin binding assays with membrane-enriched fractions from rat brain. Urethane did not alter $[^3]$H]ketanserin binding in the ex vivo assay. In the in vitro assay, urethane, chloral hydrate, and its active metabolite 2,2,2-trichloroethanol produced slight reductions (of 16%, 9%, and 18%, respectively) of $[^3]$H]ketanserin binding. These studies suggest that anti-serotonergic effects of urethane and chloral hydrate may not be mediated by a blockade of 5-HT$_2$ receptors.

Keywords: Anesthetics, chloral hydrate, 5-hydroxytryptamine (5-HT), 5-HT$_2$ receptor, $[^3]$H]ketanserin binding, urethane.

Recent evidence suggests that several general anesthetics have profound anti-serotonergic effects in the rat neocortex in vivo. Urethane, chloral hydrate, and diethyl ether all reduce or abolish the (non-cholinergic) type of electrocorticographic activation that depends on a serotonin (5-hydroxytryptamine, 5-HT) input from the raphe nuclei to the neocortex (Dringenberg and Vanderwolf, 1995).

Using the in vitro rat aortic ring preparation, it was shown that urethane strongly reduces the contractile responses to bath application of 5-HT, whereas contractile responses to noradrenaline were only slightly affected (Dringenberg et al., 1995). Thus, the action of urethane to antagonize amine
effects appears to have some specificity for the 5-HT system. The effects of urethane were not due to a blockade of calcium channels.

Contractile responses of the rat aorta to 5-HT are mediated by 5-HT₂ receptors (Cohen, 1988). Since 5-HT₂ binding sites also play a key role in serotonergic cortical activation in urethane anesthetized rats (Neuman and Zebrowska, 1992), it may be that urethane blocks 5-HT₂ receptors in both the aorta and the neocortex (see Dringenberg et al., 1995). In the present experiment, we investigated this possibility using ex vivo and in vitro binding assays for labelling of central 5-HT₂ receptors in rat brain with [³H]ketanserin (Leysen et al., 1982). Specifically, we tested whether urethane, chloral hydrate, and its active metabolite, 2,2,2-trichloroethanol may inhibit binding of [³H]ketanserin to 5-HT₂ receptors.

Materials and methods

Experiment 1: Ex vivo binding

Urethane pretreatment: Twenty-four adult male Long-Evans rats (330–400 g) were used. Rats (n = 18) were anesthetized with urethane (Sigma Chemical Co.; dissolved in saline, 250 mg/ml), administered in three doses of 500 + 300 + 250 mg/kg (i.p.) at 20 min. intervals to make a final drug concentration of 1250 mg/kg. The remaining rats (n = 6) received three i.p. injections of saline in volumes equivalent to those of the urethane injections. At each of 2, 4, and 8 hours after the last injection, urethane (n = 6) and saline (n = 2) pretreated rats were stunned with a blow to the neck and decapitated. The brain was rapidly removed, washed in ice-cold saline for 60 s, and the neocortex and hippocampal formation were separated from the rest of the brain. Both cortex (neocortex + hippocampus) and the rest of brain were frozen in 2-methylbutane over solid carbon dioxide before being packed separately in aluminum foil and stored at -70°C until the binding experiment was performed.

Receptor binding: Preparation of membrane-enriched fractions was performed as outlined in Goodnough and Baker (1994a). Briefly, cortex or rest of brain tissue was homogenized in 10 volumes of 50 mM Tris-HCl buffer (pH 7.5). Subsequently, the tissue was centrifuged at 22,000 g twice for 17 min. The resulting tissue pellet was resuspended in 10 volumes as per original weight and used for the binding experiment.

Single point binding was performed with a concentration of [³H]ketanserin (1 nM) near the Kᵦ value determined previously (Goodnough and Baker, 1994a). Incubations were performed at 37°C for 15 min and were conducted in triplicate using volumes of 1 ml. Subsequently, the tissue was rapidly filtered and washed with ice-cold Tris buffer (5 ml). Cold mianserin (1.0 μM) was used to define nonspecific binding of [³H]ketanserin (for details see Leysen et al., 1982; Goodnough and Baker, 1994a).

Experiment 2: In vitro binding

Receptor binding: Membrane preparations from rat whole cortex (not including hippocampus) were obtained as described above. Tissue was pre-incubated for 15 min with either urethane, chloral hydrate, or 2,2,2-trichloroethanol (all at a concentration of 1 mg/ml, n = 6 for each drug). Subsequently, [³H]ketanserin (1 nM) was added and receptor binding was performed as outlined above. Protein determinations were carried out using the procedure of Lowry et al. (1951).

Statistical analysis

Data are presented as mean ± S.E.M. Analyses of variance and Neuman-Keul's tests were performed using the software package CLR Anova (Version 1.1; Clear Lake Research Inc.).