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Interaction of the Competitive AMPA Receptor Antagonist NBQX With Hexobarbital

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DALL, V., U. ØRNTOFT, A. SCHMIDT AND L. NORDHOLM. *Interaction of the competitive AMPA receptor antagonist NBQX with hexobarbital.* PHARMACOL BIOCHEM BEHAV 46(1) 73–76, 1993.—IP administration of hexobarbital to rats caused a mean sleeping time of 93.6 min (SD 21.5). IV infusion of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*f*)quinoxaline (NBQX) at a dose rate of 0.11 mg/kg/min starting 30 min after administration of hexobarbital prolonged the mean sleeping time to 332.2 min (SD 74.9). Dose rates of 0.33 and 1.10 mg/kg/min prolonged the mean sleeping times to 176.4 min (SD 33.3) and 444.1 min (SD 72.0), respectively. Measured 180 and 450 min after the start of the study, there were no differences in the plasma concentrations of hexobarbital in groups receiving hexobarbital alone compared to groups receiving the high-dose rate of NBQX starting 30 min after administration of hexobarbital. The present results demonstrate that by IV infusion NBQX dose dependently prolonged the sleeping time of hexobarbital. There were no indications off interactions on hexobarbital elimination of either isomer. It is therefore likely that NBQX acts synergistically with hexobarbital to depress the central nervous activity.

AMPA receptors NBQX NBQX Hexobarbital Interaction Sleeping time Rat

2,3-DIHYDROXY-6-NITRO-7-SULFAMOYL-BENZO(*f*)-QUINOXALINE (NBQX) is a potent and specific competitive antagonist of glutamate at the D,L-α-amino-3-hydroxy-5-methyl-4-isoxalonepropionic acid (AMPA) receptor site (15). Besides being a neuroprotectant in a variety of models of cerebral ischaemia (2,3,5,8,11,15), NBQX has been shown to induce CNS depression and anaesthesia when injected directly into the cerebral ventricles (H. Klitgaard, personal communication) or by IV administration of high doses to rodents (R. K. Hjortkjaer, personal communication). It is well known that barbiturates combined with other CNS depressants, like chlorpromazine, morphine, and diazepam (13), will cause severe depression and significantly prolong the duration of sleep in laboratory animals. Glutamate antagonists acting on the NMDA site are also known to potentiate anaesthetics (4,14, 17). The metabolism of hexobarbital is affected by a range of cytochrome P450 inhibitors like *S*-mephenytoin in man (7) and doxapram and psoralens in rodents (1,6,9).

The purpose of the present study was to demonstrate whether NBQX affects the duration of sleep after administration of hexobarbital to rats and whether such an effect is mediated by inhibition of hexobarbital elimination or by direct synergistic effect on the CNS.

METHOD

Female Sprague–Dawley rats (173–185 g) purchased from Møllegårds Avlslaboratorium ApS., Ejby, Denmark, were used in the experiment. Rats were allocated with respect to body weight into five groups of five and one group of six taken alternately into study. Animals were fasted overnight and weighed before the study. Rats were anaesthetized with hexobarbital (100 mg/kg, IP) and surgically prepared with catheters in the jugular vein for drug administration and in the carotid artery for blood sampling at the termination of the study. The trachea was cannulated to facilitate respiration.

Exactly 30 min after IP administration of hexobarbital, each rat was treated by IV infusion of 3.0 ml/h of 0.9% saline (Group 1), NBQX 0.4 mg/ml (Group 2), NBQX 1.2 mg/ml (Group 3), NBQX 4.0 mg/ml (Groups 4–6). For each rat in Groups 1, 2, 3, and 4, the time from start of infusion of test substance until reappearance of the righting reflex was registered whatever the result would be. If reappearance of the righting reflex for rats in Group 5 failed within 150 min of infusion (180 min after administration of hexobarbital), the study was terminated at that time. For rats in Group 6, the time from termination of 150-min infusion of NBQX until reappearance of the righting reflex was registered. Blood samples for determination of NBQX were taken at reappearance of the righting reflex (Groups 1–4 and 6) or at termination after 150 min of infusion of NBQX despite reappearance of righting reflex (Group 5). Blood samples for determination of hexobarbital were taken at the end of the study for Groups 4 and 5. Plasma was prepared (EDTA, 3,000 rpm, 15 min) and the samples analysed for contents of NBQX by high-performance liquid chromatography (12). The concentration of

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hexobarbital (*R* and *S*) was measured based upon a well-known method (18). Pentobarbital was used as an internal standard (first peak). The results were compared to plasma concentrations of hexobarbital in groups of five hexobarbital-dosed rats after 180 and 450 min, respectively. The latter time was the mean sampling time for Group 4. The following test substances were used: hexobarbital (Mecobenzon, Copenhagen, Denmark), NBQX Na batch TU 1108.1.10 (Novo Nordisk, Pharmaceuticals Research, Måløv, Denmark), and the placebo with 5% Kollidon PF12 (BASF) and 4% glucose (Novo Nordisk, Pharmaceuticals Division). The results are presented as means and SDs. The statistical significance of the results was assessed using one-way analysis of variance (SAS System: General Linear Models Procedure and Multiple Comparisons of Least Squares Means, SAS Institute, Cary, NC) or Kruskal-Wallis test (SAS System: Nparlwayprocedure, SAS) if Bartlett's test showed lack of equality of variances at $\alpha = 0.05$.

RESULTS

The sleeping times and plasma concentrations of NBQX are shown in Table 1. IV infusion of NBQX dose dependently prolonged the sleeping time compared to the group receiving 0.9% saline at the same dose rate (3 ml/h). IV infusion of NBQX 0.4 mg/ml (Group 2, $n = 5$) corresponding to a mean dose rate of 0.11 mg/kg/min (SD 0.00) and a mean total dose of 11.33 mg/kg (SD 1.57) significantly ($p < 0.05$) prolonged the mean sleeping time to 132.2 min (SD 14.9) compared to 93.6 min (SD 21.5) for the 0.9% saline group. The mean plasma concentration at termination of study was 2.3 μg/ml (SD 0.4). The intermediate dose of 1.2 mg/ml (group III, $n = 5$) corresponding to a mean dose rate of 0.33 mg/kg/min (SD 0.00) and a mean total dose of 48.95 mg/kg (SD 11.32) significantly ($p < 0.01$) prolonged the mean sleeping time to 176.4 min (SD 33.3). The mean plasma concentration at termination of study was 7.5 μg/ml (SD 1.8). The high dose of 4.0 mg/ml (Group 4, $n = 6$) corresponding to a mean dose rate of 1.10 mg/kg/min (SD 0.01) and a mean total dose of 457.48 mg/kg (SD 83.32) significantly ($p < 0.001$) prolonged the mean sleeping time to 444.1 min (SD 72.0, $n = 4$). The mean plasma concentration at termination of study was 148.3 μg/ml (SD 48.9). Two rats from this group (IV) died during the study period. The plasma concentration of NBQX at death was 240.3 and 490.5 μg/ml, respectively.

IV infusion of the high dose of NBQX, 4.0 mg/ml, to a group of rats (Group 5, $n = 5$) corresponding to 1.13 mg/kg/min (SD 0.02) with stop of infusion after 150 min (180 min from administration of hexobarbital) resulted in a total dose of 169.14 mg/kg (SD 2.52). The mean plasma concentration at termination of study was 121.7 μg/ml (SD 23.6).

IV infusion of the high dose of NBQX 4.0 mg/ml to another group of rats (VI, $N = 5$) corresponding to 1.12 mg/kg/min (SD 0.02) with stop of infusion after 150 min (180 min from administration of hexobarbital) resulted in a total dose of 167.83 mg/kg (SD 2.95). The time to reappearance of the righting reflex was subsequently 57.4 min (SD 16.20). The mean plasma concentration at termination of study was 11.9 μg/ml (SD 5.3). The concentrations of hexobarbital are shown in Fig. 1. The concentrations for Groups 5 and 4 were not significantly different ($\alpha = 0.05$) from the plasma concentrations of hexobarbital in groups of five hexobarbital-dosed rats after 180 and 450 min, respectively.

DISCUSSION

The present results demonstrate that by IV infusion NBQX dose dependently prolonged the sleeping time of hexobarbital.

TABLE 1

DURATION OF SLEEP AND PLASMA CONCENTRATIONS OF NBQX AT TERMINATION OF STUDY

Group	Treatment	Duration of Sleep (min)	NBQX Conc (μg/ml)	*n*
1	0.9% saline, 3 ml/h	93.6 (21.5)	–	5
2	NBQX 0.4 mg/ml, 0.11 mg/kg/min	132.2 (14.9)*	2.3 (0.4)	5
3	NBQX 1.2 mg/ml, 0.33 mg/kg/min	176.4 (33.3)†	7.5 (1.8)†	5
4	NBQX 4.0 mg/ml, 1.10 mg/kg/min	444.1 (72.0)‡	148.3 (48.9)‡	4§
5	NBQX 4.0 mg/ml, 1.13 mg/kg/min for 150 min	Not recorded	121.7 (23.6)‡ (150 min)	5
6	NBQX 4.0 mg/ml, 1.12 mg/kg/min for 150 min	237.4 (16.2)‡	11.9¶ (5.3)†	5

Duration of sleep from administration of hexobarbital 100 mg/kg i.p. to reappearance of the righting reflex and plasma concentrations of NBQX at termination of study. The treatment was started exactly 30 min after administration of hexobarbital.

Results are expressed as Means (S.Dev)

§Two rats died during the study period.

¶Measured reappearance of righting reflex.

*, † and ‡: Significant differences ($p < 0.05$, $p < 0.01$ and $p < 0.001$) compared to the 0.9% saline group values (duration of sleep) or the low dose group (NBQX conc.). (Kruskal-Wallis test)

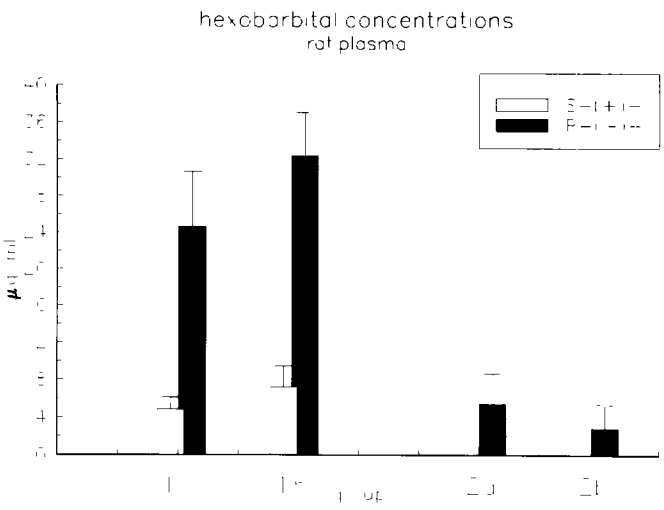

FIG. 1. (1a). Mean and SD of plasma concentrations of hexobarbital in a group (5) dosed with hexobarbital 100 mg/kg IP and infusion of NBQX at a mean dose rate of 1.13 mg/kg/min from 30–180 min after administration of hexobarbital. Blood samples at 180 min. (1b). As for 1a but no administration of NBQX. Blood samples were taken after 180 min. (2a). Mean and SD of plasma concentrations of hexobarbital in a group (4) dosed with hexobarbital 100 mg/kg IP and infusion of NBQX at a mean dose rate of 1.10 mg/kg/min from 30 min after administration of hexobarbital to death or reappearance of righting reflex. Blood samples were taken at that incidence. (2b). As for 2a but no administration of NBQX. Blood samples were taken after 450 min (mean sampling time for 2a). *S*-(+)-Concentrations were around the detection limit (0.2 μg/ml) in Groups 2a and 2b.

There is no indication of interactions on hexobarbital elimination of either isomer. It is therefore likely that NBQX acts synergistically with hexobarbital to depress the central nervous activity. In fact, such a synergistic activity could explain that in the present low-infusion-rate group (2) a reappearance of the righting reflex took place 132 min after hexobarbital administration, at which time plasma concentration of NBQX was approximately 2 μg/ml. In contrast, the plasma concentration of NBQX was approximately 12 μg/ml when righting reflex took place in the extended high-perfusion group (6) at 237 min after barbiturate administration. NBQX has been shown to synergize with another anaesthetic, halothane, as NBQX reduced the minimum alveolar concentration required for halothane in a dose-dependent manner (10). Glutamate antagonists are already used in the clinic as dissociative anaesthetics, but these drugs like ketamine acting on the NMDA site exhibit psychomimetic side effects (17). NBQX has been examined for substitution in rats discriminating another non-competitive NMDA antagonist, phencyclidine (PCP), from no drug (16). NBQX did not exhibit PCP-like effects in these studies predicting that psychomimetic symptoms and abuse liability should not be expected following administration of AMPA antagonists to humans. NBQX also failed to show morphine-like effects in a similar experiment. AMPA antagonists may therefore be a future alternative to currently used dissociative anaesthetics.

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