INHIBITORY ACTION OF THE COMBINATION OF AMINOPYRINE AND SECOBARBITAL ON EEG ACTIVATION, NEOCORTICAL RECRUITING AND AUGMENTING RESPONSES IN RABBITS

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Accepted December 6, 1979

Abstract—The effect of the combination of aminopyrine and secobarbital at a molar ratio of 2:1, the mixture and molecular compound, on rabbit EEG activation was examined. Secobarbital 40 mg/kg p.o. elevated threshold voltages in the neocortical and hippocampal EEG activation by high frequency electrical stimulation of the midbrain reticular formation (MRF), nucleus centralis medialis (CM) of the thalamus, and posterior hypothalamus (PHA) by 40, 40 and 80%, respectively. Aminopyrine 80 mg/kg p.o. alone did not affect the arousal responses. The molecular compound 120 mg/kg p.o. has more potent and long-lasting actions in inhibiting the arousal responses induced by the stimulation of MRF (80%) and CM (50%) than with secobarbital alone. The inhibitory action of the mixture 120 mg/kg p.o. on the PHA-arousal response (40%) was significantly weaker than that of the molecular compound (80%). Secobarbital and the molecular compound slightly inhibited the neocortical augmenting and recruiting responses. Our findings suggest that although aminopyrine exerts a synergistic effect with secobarbital in inhibiting the EEG activation produced by MRF stimulation, in inhibiting the PHA-arousal response, there is no synergistic effect of the two drugs when they were given as the mixture. Moreover, the molecular compound rather than the mixture has a more potent inhibitory action on the EEG activation, particularly with PHA stimulation.

Aminopyrine shows analgesic effects, although it has excitatory actions on the central nervous system (1). One of the barbiturates, secobarbital, is a short-acting hypnotic. The combination of the two drugs exhibits strong analgesic actions similarly to the combination of aminopyrine and barbital (2). In fact, we have unpublished data showing that when 2 molar aminopyrine and 1 molar secobarbital were simultaneously given p.o. as the mixture, they showed more potent analgesic effects than each of these drugs, and that the combination consisting of the molecular compound in which aminopyrine was linked by a hydrogen bond to secobarbital at the molar ratio of 1:1 and another 1 molar aminopyrine, exerted more potent analgesic actions than the mixture.

A potentiation of analgesic action by the combination may be due to the finding that aminopyrine affects the absorption and metabolism of secobarbital (3, 4), resulting in high blood levels of these drugs. However, there is no precise electrophysiological analysis on
the action sites of the combination of aminopyrine and secobarbital in the brain.

The present investigation was designed to determine the sites and mode of actions of these combinations in the rabbit brain using the EEG technique.

MATERIALS AND METHODS

The drugs used in this experiment were as follows: aminopyrine (Maruko Pharmaceutical Co., Tokyo), secobarbital, the mixture, and molecular compound. Secobarbital and the molecular compound were gifts from Grelan Pharmaceutical Co., Tokyo. The molecular compound consisted of aminopyrine and secobarbital at the molar ratio of 1:1. In a previous report we showed that the combination of these two drugs is the best at the molar ratio of 2:1 in experimental analgesic and toxic effects among various molar ratio (4). Therefore, another 1 molar of aminopyrine was added to 1 molar of the molecular compound, and this combination was referred to as the molecular compound, in the present report. Another combination, the mixture, consisted of 2 molar aminopyrine and 1 molar secobarbital. The drugs of four groups were suspended in 0.5% carboxymethyl cellulose just before administration, and the suspension was given p.o. to chronically electrode-implanted rabbits which had been fasting for 24 hours.

White male rabbits weighing 2.5 to 3.5 kg were anesthetized with sodium pentobarbital (25 mg/kg i.v.) and fixed on a stereotaxic apparatus (Todai Noken type). Stereotaxic coordinates were obtained from the brain atlas of Sawyer et al. (5). Bipolar stainless-steel electrodes (0.2 mm in diameter, a distance of 0.3 to 0.5 mm between two tips) were used to stimulate and obtain recordings of electrical activity from various subcortical brain areas. Subcortical recordings were obtained from the hippocampus (A:-6.0, L: 6.0, H: 5.0) and amygdala (A: 1.5, L: 5.0, H: -5.0). Stimulating electrodes were placed in the midbrain reticular formation (A:-9.0, L: 3.0, H:-3.0, MRF), posterior hypotalamus (A:-1.0, L: 1.5, H:-3.0, PHA), nucleus centralis medialis (A:-5.0, L: 2.0, H:-1.0, CM), nucleus ventralis anterior (A:-2.0, L: 3.5, H:-0.5, VA), and nucleus ventralis posterolateralis (A:-4.0, L: 4.5, H:-1.0, VPL) of the thalamus. In order to avoid injury to the animals, the stimulating electrodes were implanted into two subcortical brain areas of the animal.

Silver bipolar electrodes (diameter of 1.0 mm) were placed on the dura mater to record neocortical EEG from the motor area (A: 0.0 to 5.0, L: 3.0) and sensory area (A:-5.0 to 0.0, L: 8.0).

Satisfactory subcortical electrode placements were established by recording of injury discharges as the electrodes entered the hippocampus and amygdala. EEG activation by high frequency electrical stimulation was used to identify MRF and PHA. Neocortical recruiting and augmenting responses produced by low frequency electrical stimulation were used to identify the thalamic nuclei. After experiments, subcortical electrode positions were histologically verified by an iron deposition technique (6).

The electrodes were connected to the connector socket which was fixed with dental cement on the skull. Iodine tincture was applied on the operated region and 100,000 units of penicillin were injected i.m. once a day for three days after operation. The animals
were used in the experiments at least a week after operation.

The EEG was recorded using a polygraph recorder (Nihon Kohden, RM-45), and electrical stimulation was applied by a pulse generator (Nihon Kohden, MSE-3R). Time course of changes of the spontaneous EEG, the arousal responses produced by high frequency electrical stimulation of MRF, CM and PHA, the recruiting response produced by low frequency electrical stimulation of CM or VA, and the augmenting response produced by low frequency electrical stimulation of VPL were observed before and after drug administration. Conditions of the electrical stimulation were as follows: a frequency, 8 or 100 Hz; a duration, 1.0 msec for 5 sec.

The effects of drugs were represented as per cent increase relative to the initial threshold voltage (before administration), and the statistical comparisons at a given time after administration were done between drugs. In addition, changes in threshold voltages were statistically analyzed for each of the drugs.

RESULTS

Effect on spontaneous EEG and behavior: When secobarbital was given p.o. in a dose of 30 mg/kg to rabbits, no marked alteration of the spontaneous EEG patterns occurred within 2 hr after the administration. However, spindles and high voltage slow waves in neocortical EEG and irregular high voltage waves in hippocampal EEG appeared 1 hr after secobarbital 40 mg/kg p.o. In a dose of 50 mg/kg p.o., neocortical EEG activity was characterized by high voltage slow waves 30 min after the administration. At this time the rabbits fell asleep. A dissociation between the EEG patterns and behavior was not observed with any dose, therefore, we decided on the dose of 40 mg/kg of secobarbital. Since the molar ratio of secobarbital and aminopyrine used in this experiment was 1:2, the dose of aminopyrine was 80 mg/kg. At this dose, aminopyrine did not affect the spontaneous EEG patterns of neocortical and subcortical regions within 2 hr after the administration while the rabbits became somewhat restless.

When the mixture 120 mg/kg of secobarbital (40 mg/kg) and aminopyrine (80 mg/kg) at the molar ratio of 1:2 was given p.o., neocortical EEG patterns were characterized by high voltage slow waves 30 min after the administration. Recovery occurred at about 3 hr. The effects of the molecular compound 120 mg/kg p.o. were exactly the same as those of the mixture. The action of secobarbital on rabbit EEG and behavior was potentiated by a combination with aminopyrine.

Effect on EEG activation produced by electrical stimulation of MRF: Figure 1 shows changes in the threshold voltage producing the arousal responses in neocortical and hippocampal EEG by electrical stimulation of MRF (100 Hz, 1 msec for 5 sec) after drug administration. The initial threshold voltage was $2.3 \pm 0.1$ V (the mean and SEM, n=21). All drugs used in these experiments, except for aminopyrine, elevated the threshold voltage. The maximal effect was found at 45 min for secobarbital and 60 min for the two combinations. The combinations significantly inhibited the arousal responses ($P<0.05$ or 0.01), and were more potent in inhibiting the EEG activation by MRF stimulation than was
secobarbital alone (P<0.10 or 0.05). Recovery was found at 2 hr for secobarbital and at 3.5 hr for the mixture and the molecular compound. Aminopyrine alone did not affect the EEG activation by MRF stimulation at any time. Therefore, we did not examine the effect of aminopyrine on the arousal responses induced by high frequency electrical stimulation.

**Fig. 1.** Effect of aminopyrine, secobarbital, the mixture and molecular compound on the threshold voltage of the neocortical and subcortical arousal responses induced by high frequency electrical stimulation of the midbrain reticular formation (MRF). Aminopyrine 80 mg/kg, secobarbital 40 mg/kg, the mixture or molecular compound at the molar ratio of 2:1, 120 mg/kg, was given orally to chronically electrode-implanted rabbits. Aminopyrine alone did not affect the threshold voltage (data not shown). Each plot shows the mean, and the vertical lines show SEM. The values in parentheses show the numbers of experiments. There were statistical increases in the threshold voltage from the corresponding initial values (*, P<0.05; **, P<0.01) and differences between secobarbital alone and the mixture or the molecular compound (a, P<0.10, b, P<0.05).

**Fig. 2.** Effect of secobarbital, the mixture and molecular compound with aminopyrine on the threshold voltage of the neocortical and subcortical arousal responses induced by high frequency electrical stimulation of nucleus centralis medialis (CM) of the thalamus. Conditions as indicated in Fig. 1. Each plot shows the mean, and the vertical lines show SEM. The values in parentheses show the numbers of experiments. There were statistically significant increases in the threshold voltage from the corresponding initial values (*, P<0.05; **, P<0.01), but there was no statistically significant difference between the drugs.
in subsequent experiments.

Effect on EEG activation produced by electrical stimulation of CM: Figure 2 shows changes in the threshold voltage causing neocortical and hippocampal EEG activation by high frequency electrical stimulation of CM (100 Hz, 1.0 msec for 5 sec) following drug administration. The initial threshold voltage in CM stimulation was 2.7±0.1 V (the mean and SEM, n=18). Secobarbital alone and the two combinations elevated the threshold voltage. The peak time was found at 1 hr in all the groups. There were no significant

![Diagram](image)

**FIG. 3.** Effect of aminopyrine, secobarbital, the mixture and molecular compound on the threshold voltage of the neocortical recruiting response induced by low frequency electrical stimulation of nucleous centralis medialis (CM) or ventralis anterior (VA) of the thalamus. Conditions as indicated in Fig. 1. Each plot shows the mean, and the vertical lines show SEM. The values in parentheses show the numbers of experiments. No statistically significant change in the threshold voltage was found in the individual drugs and between the drugs.

![Diagram](image)

**FIG. 4.** Effect of aminopyrine, secobarbital, the mixture and molecular compound on the threshold voltage of the neocortical augmenting response induced by low frequency electrical stimulation of nucleous ventralis posterolateralis (VPL) of the thalamus. Conditions as indicated in Fig. 1. Each plot shows the mean, and the vertical lines show SEM. The values in parentheses show the numbers of experiments. No statistically significant change in the threshold voltage was found in the individual drugs, but there was a significant difference between the mixture and molecular compound (b, P<0.05).
differences in the inhibitory action between secobarbital alone and the mixture or the molecular compound, although the combinations appear to be more potent than secobarbital alone. The inhibitory action of the combinations on the CM-arousal response was weaker than that on the MRF-arousal response. Recovery was found at 3 hr for secobarbital, and its inhibitory action on the CM-arousal response seems to last longer than that on the MRF-arousal response.

Effect on recruiting and augmenting responses produced by electrical stimulation of CM or VA and VPL: Figures 3 and 4 show the effect on the recruiting and augmenting responses, respectively. The initial threshold voltage was $3.5 \pm 0.1$ V (the mean and SEM, $n=21$) for the recruiting response and $3.4 \pm 0.2$ V (the mean and SEM, $n=17$) for the augmenting response. The recruiting and augmenting responses were scarcely affected by any of the drugs used. Since secobarbital alone and the molecular compound showed a slightly inhibitory action on the augmenting response, there was a significant difference in inhibiting the augmenting response between the mixture and the molecular compound ($P<0.05$).

Effect on EEG activation produced by electrical stimulation of PHA: Figure 5 shows changes of the threshold voltage produced by the arousal response in the neocortical and hippocampal EEG by electrical stimulation of PHA (100 Hz, 1.0 msec for 5 sec) after drug administration. The initial threshold voltage in PHA stimulation was $2.6 \pm 0.1$ V (the mean and SEM, $n=13$). All the drugs, except for aminopyrine, significantly inhibited the neocortical and hippocampal EEG activation by PHA stimulation. The maximal effect was found at 1 hr for all the drugs. Secobarbital alone and the molecular compound have a more potent inhibitory action than the mixture ($P<0.10$ or 0.05). The inhibitory effect of secobarbital on the PHA-arousal response appears to be more potent and long-lasting.

Fig. 5. Effect of secobarbital, the mixture and molecular compound with aminopyrine on the threshold voltage of neocortical and subcortical arousal response induced by high frequency electrical stimulation of the posterior hypothalamus (PHA). Conditions as indicated in Fig. 1. Each plot shows the mean, and the vertical lines show SEM. The values in parentheses show the numbers of experiments. There were statistically significant increases in the threshold voltage from the corresponding initial values (*, $P<0.05$; **, $P<0.01$), and significant differences between the mixture and molecular compound (a, $P<0.10$; b, $P<0.05$).
than that on the MRF-arousal response. Statistically significant differences were found between the molecular compound and the mixture during 45 to 90 min after the administration.

**DISCUSSION**

The molecular compounds linked by hydrogen bond, pyrabital (barbital and aminopyrine) and secopyrabital (secobarbital and aminopyrine) used in this present report, had been considered to dissociate easily into the individual drugs in aqueous solution (7). The dissociation may, however, occur completely when a molecular compound is suspended in a solution of carboxymethyl cellulose, but a complex of aminopyrine and secobarbital may be formed in an aqueous solution when they are dissolved simultaneously (8). Thus, the effects of the chemical difference between the mixture and molecular compound in the stomach are unknown.

In the present report, we demonstrated pharmacologically a difference in inhibition of the EEG activation produced by electrical stimulation of particularly PHA among aminopyrine, secobarbital and their combinations. The inhibitory action of the mixture on the PHA-arousal response was weaker than that of secobarbital alone and the molecular compound. A similar pattern was found in the neocortical augmenting response. This may be due to the finding that the inhibitory action of the mixture on the PHA-arousal response is decreased by the excitatory effect of aminopyrine. Therefore, aminopyrine may decrease the inhibitory actions of secobarbital on the EEG activation and neocortical augmenting response when given in combination with secobarbital.

The molecular compound had a significantly more potent action in inhibiting the PHA-arousal response than the mixture. If the molecular compound had been dissociated easily into the individual drugs in the stomach, similar inhibitory effects would have been apparent with both the molecular compound and the mixture. Therefore, we assume that the molecular compound itself is absorbed and then affects the PHA-arousal response. In our previous report (4), the molecular compound of aminopyrine and secobarbital was shown to inhibit the writhing response induced by acetic acid in mice and the pseudo-affective responses induced by bradykinin in rats more strongly than the mixture. These findings may be explicable by the difference in inhibitory action to the PHA-arousal response between the molecular compound and the mixture. The molecular compound inhibited the EEG activation by electrical stimulation in the order of PHA, MRF and CM. Since the PHA is a region with an incomplete bloodbrain barrier, the transport of the molecular compound to PHA appears to be faster than that to the other brain regions. This may explain the potent inhibitory action of the molecular compound, the mixture and secobarbital alone on the PHA-arousal response.

Aminopyrine was shown to have a biphasic effect on the central nervous system. Ban (9) has shown that aminopyrine 50 mg/kg i.v. inhibits a neocortical recruiting response and elevates the threshold voltage required for the EEG activation by MRF stimulation. We did, however, detect a slightly excitatory effect rather than inhibitory actions of aminopyrine
Barbiturates inhibit the EEG activation induced by electrical stimulation of MRF and PHA (10). In the present experiment, we confirmed the inhibitory actions of secobarbital on the neocortical and subcortical arousal responses induced by electrical stimulation of MRF and especially PHA. Inhibitory actions of the combinations with aminopyrine appear to be based on the actions of secobarbital, because aminopyrine showed slight, excitatory effects only. With the prolongation of gastric emptying time by simultaneous administration of aminopyrine and secobarbital, aminopyrine may increase blood secobarbital levels when given in combination (4).

In conclusion, the inhibitory actions of the combination of aminopyrine and secobarbital to EEG activation may probably be based on those of secobarbital, and there is a statistically significant difference in inhibition of the PHA-arousal response, in particular between the molecular compound and mixture.

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