EFFECTS OF AMINOPYRINE AND PHENYL BUTAZONE ON THE ACTIVITY OF NEURONS IN THE TRIGEMINAL NUCLEI AND HYPOTHALAMUS

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Informations for the neural mechanism of pain sensation are still insufficient. Haugen and Melzack (1, 2) have shown that the afferent fibers most closely related to pain perception caused by electrical stimulation of the tooth pulp project to the sensory cortex through the trigemino-bulbo-thalamic tract, but not via the trigeminal lemniscus. The significance of participation of the thalamic intralaminar nuclei in pain mechanism has been emphasized by Gangloff and Monnier (3) and Albe-Fessard and Kruger (4). However, the concept of a specific pain center in the brain is considered to be totally inadequate, since the thalamus, hypothalamus, brainstem reticular formation and parietal cortex are all implicated in pain perception (5).

In the previous paper (6), it was presumed that 1) the hypothalamus formed closed circuits with other brain structures, and 2) the circulation of impulses within these circuits might contribute to the central regulation of sensory mechanism. In the present experiments, the effects of aminopyrine and phenylbutazone on the evoked potentials in the sensory cortex and posterior hypothalamus as well as on the responses of unit discharges in the posterior hypothalamus and trigeminal nuclei to afferent electrical stimulation of the inferior alveolar nerve were studied in attempt to find the mode of action of these analgesics in the central nervous system.

METHODS

About 50 adult cats of either sex, weighing from 2.5 to 3.5 kg, were used. Details of the experimental procedures were described in other papers which have already been reported (6, 7). Encephale isolé preparation of the animal was fixed on the stereotaxic instrument, and kept in sound-proof room maintained at 28°C.

Recording procedures: A silver ball tipped electrode of 0.5 mm in diameter was used for recording of spontaneous EEG and evoked potentials on the left sensory cortex (Area II of Woolsey and Fairman (8)), and a silver wire electrode of 0.3 mm in diameter was employed for recording of spontaneous EEG from the left posterior hypothalamus (A: 10.0, L: 0.5 to 1.0, H: -3.0 to -4.0) according to the topographic map of Snider and Niemer (9). A glass capillary microelectrode filled with 3 M KCl containing fast green
FCF (electrical resistance: 6–15 MΩ) was used for recording of unit discharges in the left posterior hypothalamus and a glass-insulated silver wire, 20 to 25 μ at the tip (electrical resistance: 0.7–1 MΩ), was used for recording of unit discharges in the right Nucleus sensorius superior n. trigemini (P: 5.0, L: 5.0, H: −4.0 to −5.5) and the right Nucleus tractus spinalis n. trigemini (P: 6.5, L: 4.5, H: −5.0 to −6.5).

A polygraph (Nihon Koden, RM-150) and an ink-writing oscillograph (Nihon Koden, WI-180-TR) were used for recordings of the spontaneous EEG and evoked potentials. The evoked potentials were observed by photographing the averaged pattern of 20 successive trials using a computer (San’ei Sokki, Mediac MC-401). The unit discharges were photographed with a long-recording camera and simultaneously recorded on an ink-writing oscillograph utilizing a train pulse generator (Nihon Koden, ST-1). The locations of recording electrodes were histologically confirmed after the termination of the experiments by the method which has been previously shown (6).

Stimulation procedures: The right inferior alveolar nerve was exposed by a ventral approach, and a pair of silver wire electrodes was twined round the nerve trunk. Single square wave pulse, 1 msec in width, 3.0–6.0 volts in intensity and in every two second interval, was applied to the inferior alveolar nerve for observation of the evoked potentials in the sensory cortex and hypothalamus and of the unit discharges in the trigeminal nuclei. The continuous stimuli consisted of 10 and 100 cps frequency, 1 msec in pulse width and 5 seconds in duration, were applied to the nerve for recording of the hypothalamic unit discharges. In addition, the intragastric infusion of 15 ml/kg of cold water (4°C) was used as the visceral stimulation.

Drug-used: Aminopyrine was dissolved in physiological saline, and phenylbutazone was suspended into 0.5% carboxymethyl cellulose solution. They were injected into the femoral vein.

Evaluation of the data: The significance of the changes in firing rate of the unit discharges was evaluated by adopting chi-square test at P=0.05.

RESULTS

1. Effects of aminopyrine and phenylbutazone on the spontaneous EEGs in the sensory cortex and hypothalamus

The intravenous injection of aminopyrine in doses less than 10 mg/kg did not affect the pattern of the spontaneous EEGs in the sensory cortex and posterior hypothalamus. About 20 seconds after the injection of 20 mg/kg of aminopyrine, their spontaneous EEGs were altered into the pattern of low voltage fast waves and the spindle bursts completely disappeared, as can be seen in Fig. 1. The spike waves were occasionally intermingled in the fast pattern. In 15 to 20 minutes after the administration of the drug, spontaneous EEGs usually recovered the pattern before the injection. Immediately after the additional injection of 30 mg/kg of aminopyrine (total dose of 50 mg/kg), spontaneous EEGs in the sensory cortex and hypothalamus turned to low voltage fast waves intermingled with spikes, and they were followed by seizure discharges which persisted simultaneously in
Effects of aminopyrine on the spontaneous EEGs in the sensory cortex (SC) and posterior hypothalamus (Hyp). Time scale: 1 second.

Effects of phenylbutazone on the spontaneous EEGs in the sensory cortex (SC) and posterior hypothalamus (Hyp). Time scale: 1 second.

Fig. 1. Effects of aminopyrine on the spontaneous EEGs in the sensory cortex (SC) and posterior hypothalamus (Hyp). Time scale: 1 second.

Fig. 2. Effects of phenylbutazone on the spontaneous EEGs in the sensory cortex (SC) and posterior hypothalamus (Hyp). Time scale: 1 second.

Both structures lasting 30 to 60 seconds. The EEGs were flattened for about 15 seconds after the cessation of the seizure discharges, and then they were restored to the low voltage fast waves intermingled with spikes. In some cases, seizure discharges reappeared two or three times within several minutes after the injection. The spontaneous EEGs recovered to the control pattern in 60 minutes after the drug administration. The EEGs of the sensory cortex and hypothalamus usually responded to aminopyrine in a parallel manner, but in some of the animals treated with aminopyrine the spike waves appeared in the hypothalamic EEG alone. During activation of the spontaneous EEGs, shivering and twitching of the skeletal muscles were occasionally observed.

Fig. 2 shows the effect of phenylbutazone on the cortical and hypothalamic EEGs. The intravenous injection of 10 mg/kg of phenylbutazone did not cause any changes in the cortical and hypothalamic EEGs in 3 of 6 animals, while in remaining 3 animals the same dose of the drug resulted in EEGs of low voltage fast waves in both structures. Within one minute after the additional administration of 20 mg/kg of phenylbutazone (total dose of 30 mg/kg), the spontaneous EEG in the hypothalamus showed low voltage fast waves, and 3 to 4 minutes thereafter the cortical and hypothalamic EEGs were altered into slow or flattened pattern. The appearance of spike waves and seizure discharges in the EEGs was not observed in any cases following the administration of phenylbutazone.

2. Effects of aminopyrine and phenylbutazone on the evoked potentials in the sensory cortex and hypothalamus

The evoked potentials in the sensory cortex and posterior hypothalamus in response
to single electrical stimulation of the contralateral inferior alveolar nerve consisted of 2 or 3 negative components. Latencies of the first component of the cortical and hypothalamic evoked potentials were 6 to 8 msec and 4 to 5 msec, respectively. In the sensory cortex, the amplitude of the first component was in the range of 50 to 200 μV and that of the second component was 200 to 500 μV. In the posterior hypothalamus, the first and second components were in the range of 60 to 150 μV and 100 to 500 μV in amplitude, respectively. These potentials were sometimes followed by the third negative component of 25 to 120 μV in amplitude.

Fig. 3 shows the effects of aminopyrine on averaged evoked potentials of 20 successive trials of single electrical stimulation to the inferior alveolar nerve in the sensory cortex and hypothalamus. During appearance of the arousal pattern in the EEG by the injection of 20 mg/kg of aminopyrine, a marked decrease in amplitude of the evoked potentials in the sensory cortex and hypothalamus was observed without showing any change in their latencies. At 10 minutes after the injection, the mean percent decrease in amplitude of the first and second components in 6 animals was 67.2 ± 4.5 (S.E.) and 49.3 ± 2.3 in the sensory cortex, and 26.8 ± 9.7 and 23.7 ± 12.2 in the hypothalamus, respectively (Table 1). At 10 minutes after the additional injection of 30 mg/kg of aminopyrine (total dose of 50 mg/kg), further reduction in amplitude of both components was observed. The mean percent decrease in amplitude of the first and second components was 80.6 ± 6.3 and 76.1 ± 11.0 in the sensory cortex and 60.9 ± 8.5 and 45.1 ± 11.0 in the hypothalamus, respectively.

Phenylbutazone in dose of 10 mg/kg markedly decreased the amplitude of the second component of the cortical evoked potential, while the first component of the potential in
both sensory cortex and hypothalamus was hardly affected by the drug, as shown in Fig. 4. At 10 minutes after the additional injection of 20 mg/kg of phenylbutazone (total dose of 30 mg/kg), the mean percent decrease in amplitude of the first component was only 10.9±6.8 in sensory cortex and 16.1±3.2 in the hypothalamus, while that of the second component was 51.2±16.4 in the former structure and 35.8±25.2 in the latter structure (Table 1).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Doses (mg/kg)</th>
<th>No. of animals</th>
<th>Percent decrease of amplitude</th>
<th>Sensory cortex</th>
<th>Posterior hypothalamus</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td>1st component</td>
<td>2nd component</td>
<td>1st component</td>
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<tr>
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<td>6</td>
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<td>49.3±2.3</td>
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<td></td>
<td>50</td>
<td>6</td>
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<td>4</td>
<td>1.4±6.6</td>
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<tr>
<td>Phenylbutazone</td>
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<td>4</td>
<td>10.9±6.8</td>
<td>51.2±16.4</td>
<td>16.1±3.2</td>
</tr>
</tbody>
</table>

Values are mean±S.E.

Table 1. Effects of aminopyrine and phenylbutazone on the evoked potentials of the sensory cortex and posterior hypothalamus in response to electrical stimulation of the inferior alveolar nerve.

3. Effects of aminopyrine and phenylbutazone on unit discharges in the posterior hypothalamus

The intravenous injection of 20 mg/kg of aminopyrine produced a slight decrease in firing rate of spontaneous unit discharges of the posterior hypothalamus in 2 of 4 animals. In remaining animals, however, the firing rate of hypothalamic unit discharges was slightly increased by the same dose of the drug. At 10 minutes after the additional injection of 30 mg/kg of aminopyrine (total dose of 50 mg/kg), the firing rate of the discharges showed a significant decrease (P<0.05) in all animals (Table 2).

It was confirmed to exhibit a significant increase in firing rate of 4 hypothalamic units in response to electrical stimulation of the inferior alveolar nerve (1 msec, 10 and 100 cps for 5 seconds) and intragastric infusion of cold water (4°C, 15 ml/kg). Fig. 5 illustrates a typical hypothalamic neuron, of which the firing rate was increased by the somato-sensory...
and visceral stimulation before the injection of aminopyrine. Ten to 15 minutes after the injection of 50 mg/kg (total dose), increasing response of the firing rate to the somatosensory stimulation was completely depressed, as shown in Figs. 5 and 6. The response of unit discharges to the visceral stimulation was also suppressed by the same dose of
aminopyrine in 3 of 4 units except one which showed no significant change in the discharges pattern.

Table 2 demonstrates the mean firing rate/sec of 4 hypothalamic units during and after electrical stimulation of the inferior alveolar nerve and after intragastric infusion of cold water, before and 10 to 15 minutes after the injection of aminopyrine. Aminopyrine in doses of 20 and 50 mg/kg completely suppressed the increase in firing rate of the unit discharges in response to the somato-sensory stimulation. The dose of 50 mg/kg also significantly depressed the increased response in the firing rate to the visceral stimulation.

The intravenous injection of 10 mg/kg of phenylbutazone caused a slight decrease in firing rate of the spontaneous unit discharges in one of 3 cases. Ten to 15 minutes after the additional injection of 20 mg/kg of phenylbutazone (total dose of 30 mg/kg), the firing rate of the discharges exhibited a significant decrease (P<0.05), as shown in Table 2.

![Figure 7](image)

**Fig. 7.** The time-course of changes in firing rate of the hypothalamic unit discharges induced by electrical stimulation (10 and 100 cps) of the inferior alveolar nerve and by intragastric infusion of cold water (4°C) before and after the intravenous injection of 30 mg/kg of phenylbutazone.

In one of 3 hypothalamic units which had been confirmed to increase in the firing rate in response to electrical stimulation of the inferior alveolar nerve and to intragastric infusion of cold water, 10 mg/kg of phenylbutazone apparently inhibited the increased response to the somato-sensory stimulation. In remaining 2 units, however, the same dose did not modify the increased response to the stimulation. Fig. 7 illustrates the time-course of changes in the firing rate of the hypothalamic unit discharges induced by electrical stimulation of the inferior alveolar nerve and by infusion of cold water before and 10 to 15 minutes after the intravenous injection of 30 mg/kg of phenylbutazone. The increased responses in the firing rate to these stimulations were completely suppressed by the dose of phenylbutazone in all 3 units (Table 2).

4. **Effects of aminopyrine and phenylbutazone on the unit discharges in the trigeminal nuclei**

In the observation of 21 units of the Nucleus sensorius superior n. trigemini, electrical stimulation of the ipsilateral inferior alveolar nerve produced an increase in firing rate of the discharges in 9 units (42.8%), the decrease in 3 units (14.4%) and no-change in 9 units (42.8%). Among 20 units of the Nucleus tractus spinalis n. trigemini, 6 units
(30%) responded to the somato-sensory stimulation with increase in firing rate, while remaining 14 units (70%) did not respond to the same procedure.

In respective 3 units of both structures tested, doses of 20 and 50 mg/kg of aminopyrine affected neither the firing rate of the spontaneous unit discharges nor the response of units to the somato-sensory stimulation (Fig. 8). The firing rate of the unit discharges in these trigeminal nuclei was not modified by the intravenous injection of 10 mg/kg of phenylbutazone, while it was slightly decreased by the injection of 30 mg/kg. However, the increased response of the firing rate of the discharges in both structures to the somato-sensory stimulation was not affected by the doses of phenylbutazone.

DISCUSSION

The physiological role of the first relay station of the spinal cord in pain sensation mechanisms has been proposed by Melzack and Wall (5). It has been reported that morphine and other narcotic analgesics block the afferent impulses of the splanchnic, phrenic and inferior cardiac nerves in the spinal cord (10, 11). Mizoguchi (12) has also demonstrated that one of main sites of blocking action of morphine on the afferent impulse from the tooth pulp is the Nucleus tractus spinalis n. trigemini, because the drug depressed the evoked potentials in the nucleus caused by tooth pulp stimulation. Sasa (13) has shown that morphine decreases the firing rate of the spontaneous unit discharges in the Nucleus sensorius superior n. trigemini and increases that in the Nucleus tractus spinalis n. trigemini.
The increased response of the unit discharges to electrical stimulation of the inferior alveolar nerve was also inhibited by morphine in the neurons of both trigeminal nuclei. In the present experiments, however, neither aminopyrine nor phenylbutazone produced any significant change in the firing rate of the spontaneous unit discharges and in the increased response of the discharges to the nerve stimulation recorded from the neurons of both trigeminal nuclei. These results indicate that these antipyretic analgesics behave differently from morphine at the first relay station of the afferent trigeminal impulses.

Fujita et al. (11) have shown that aminopyrine depresses the conduction of repetitive impulses through the Nucleus ventralis postero-lateralis of the thalamus. Monnier et al. (14) have also demonstrated that aminopyrine suppresses the EEG arousal response to repetitive stimulation of the midbrain reticular formation and of the posteroventral hypothalamus. In the present experiments, aminopyrine resulted in a decrease in amplitude of the evoked potentials in the sensory cortex and posterior hypothalamus induced by the trigeminal stimulation. The drug also reduced the firing rate of the spontaneous unit discharges in the posterior hypothalamus, and it depressed the increased response of the unit discharges to electrical stimulation of the inferior alveolar nerve and to intragastric infusion of cold water.

On the other hand, Ban (15, 16) has reported that intravenous injection of aminopyrine produces the arousal pattern of the cortical and hypothalamic EEGs mixed with spike waves and seizure discharges in some cases, and the present experiments have also confirmed his findings. These results offer an assumption that aminopyrine is possessed of a stimulating action on the central nervous system. As shown by many investigators (17-19), repetitive stimulation of the brainstem reticular formation mostly inhibits the amplitude of the cortical evoked potentials, while it activates the cortical spontaneous EEG. Therefore, the depression of the evoked potentials in the sensory cortex and hypothalamus induced by aminopyrine might be related with the stimulating effect of the drug.

The intravenous administration of 10 mg/kg of phenylbutazone did not markedly affect the cortical and hypothalamic EEGs. In larger doses of the drug, the EEGs altered into slow or flattened pattern but spike waves or seizure discharges were never encountered in both structures. Phenylbutazone diminished markedly the amplitude of second component of the evoked potentials and the firing rate of the spontaneous unit discharges in the posterior hypothalamus. The increased responses of the unit discharges to electrical stimulation of the inferior alveolar nerve were also suppressed by the drug.

Therefore, it is presumed that aminopyrine and phenylbutazone may act on the hypothalamic neurons and/or on the closed circuits of impulses including the hypothalamus, which may contribute to the central regulation of sensory mechanisms as described in the previous paper (6).

**SUMMARY**

Effects of aminopyrine and phenylbutazone on the spontaneous EEGs and evoked potentials in the sensory cortex and posterior hypothalamus and on the responses of unit
discharges in the posterior hypothalamus and trigeminal nuclei to electrical stimulation of the inferior alveolar nerve were studied in the encephale isolé preparations of cats.

1. The spontaneous EEGs in the sensory cortex and hypothalamus turned to the arousal pattern after the intravenous injection of aminopyrine. Larger doses of aminopyrine produced spike waves and seizure discharges in the EEGs. The intravenous injection of phenylbutazone did not markedly change the cortical and hypothalamic EEGs but larger doses of the drug produced slow or flattened pattern in both structures.

2. Aminopyrine diminished the amplitude of the first and second components of the evoked potentials in the sensory cortex and hypothalamus induced by stimulation of the inferior alveolar nerve, while phenylbutazone showed a marked decrease in amplitude of the second component alone.

3. Aminopyrine and phenylbutazone reduced the frequency of the spontaneous unit discharges in the posterior hypothalamus, and they depressed the increased response to electrical stimulation of the inferior alveolar nerve or intragastric infusion of cold water.

4. In the Nucleus tractus spinalis n. trigemini and Nucleus sensorius superior n. trigemini, aminopyrine and phenylbutazone did not significantly change the firing rate of the spontaneous unit discharges and the increased response of the discharges to electrical stimulation of the inferior alveolar nerve.

REFERENCES

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