Abstract—The mode of action of nefopam, a novel analgesic, on the splanchnic afferent pathway was investigated using electrophysiological methods. Nefopam (2.5 and 5.0 mg/kg, i.v.) caused arousal patterns in spontaneous rabbit EEG. In intact cats, nefopam (1.0, 2.5 and 5.0 mg/kg, i.v.) suppressed the evoked potentials recorded from the posterior sigmoid gyrus of the cortex, N. ventralis posterolateralis and N. centralis medialis of the thalamus and the ventrolateral funiculus of the spinal cord following splanchnic nerve stimulation without inhibiting potentials in the thalamo-cortical pathways. These depressant effects were not antagonized by a narcotic antagonist, levallorphan (0.5 mg/kg, i.v.). The inhibitory effect of nefopam on the spinal potential evoked by splanchnic nerve stimulation was not observed in spinal cats (C1–C2 transection) and pentobarbital-anesthetized cats. These results suggest that nefopam may inhibit the splanchnic afferent pathways in the spinal cord by reinforcing descending inhibitory systems originating in the supra-spinal structure, in a manner which differs from that seen with morphine.

Nefopam hydrochloride (5-methyl-1-phenyl-1,3,4,6-tetrahydro-5H-2,5-benz [f] oxazocine hydrochloride), the structure of which is in Fig. 1, is a novel heterocycle unrelated chemically to other analgesics.

According to Conway and Mitchell (1), nefopam was found to be at least as potent as codeine phosphate in the p-phenylquinone writhing and the monkey tail flick test, and to possess an anti-nociceptive profile distinct from those of other currently available analgesic agents. Although active in the p-phenylquinone induced writhing test, the mouse hot plate test, the Haffner tail clamp test, the cat tooth pulp test and the monkey tail flick test, nefopam was clearly unlike narcotics in that it had no effect in the mouse radiant heat test and in the rat Randall-Selitto test. Moreover, the anti-nociceptive action of nefopam in the monkey tail flick test was not blocked by naloxone, and no cross-tolerance appeared to exist between nefopam and morphine in the mouse hot plate test.

This compound is also unlike narcotic-antagonist analgesics in that the anti-nociceptive activity of morphine was not antagonized in the mouse radiant heat test.
In addition, it is different from the anti-inflammatory analgesics because it was inactive in the rat Randall-Selitto test and did not inhibit prostaglandin synthesis, except at very high concentrations.

Nefopam, unlike morphine, lacked potential for tolerance development and proved to have low dependence liability in animal models (2).

In therapeutic trials, analgesia was achieved without serious side effects usually in postoperative patients and the relative potency of nefopam to morphine was shown to be about 0.2 to 0.6 (2). However, the mode of action of this compound remains unknown.

The present paper describes studies on the mode of action of nefopam using electrophysiological techniques and the findings were compared with the data on morphine.

MATERIALS AND METHODS

I) Electroencephalographic studies

Three male white rabbits weighing 2.7 to 3.4 kg were used. The implantation of EEG electrodes was performed under pentobarbital anesthesia (30 mg/kg, i.v.). The electrodes were placed in the sensorimotor area of the cortex (SMC), hippocampus (HPC) (P:3, L:4, H:5) and amygdala (AMY) (A:2, L:7, H:-5), according to a stereotaxic atlas of Sawyer et al. (3). The electrodes used were the bipolar silver ball electrodes (diameter of 1.0 mm) for recording the cortical EEG and the bipolar concentric electrodes made from insulated stainless steel pipe except the tip (outside diameter of 0.5 mm, distance of 1.0 mm between two electrode tip) for recording the subcortical EEG. The electrodes were connected to the miniature socket, which was fixed with dental cement to the skull. The experiments were carried out later than 3 weeks after implantation.

II) Evoked potential studies

Forty adult cats of both sexes weighing 2.2 to 4.8 kg were used. Xylocaine jelly was frequently applied to the tracheal cannula, ear canals, pressure points and the entire surgical areas throughout the entire experiment. Animals underwent the surgery after anesthetization with ether and were then immobilized with gallamine triethiodide, artificially ventilated and placed in a stereotaxic apparatus (Todai Noken Type). The brain and the spinal cord were exposed at appropriate locations. In some experiments, spinal cats (transected at the C1–C2 level) and pentobarbital-anesthetized cats (30 mg/kg, i.p.) were used. The bipolar concentric electrodes for the subcortical stimulation and recording were inserted stereotaxically according to a stereotaxic atlas of Jasper and Ajmone-Marsan (4).

1) Effects on afferent pathway of the splanchnic nerve: The left splanchnic nerve was exposed and ligated at its peripheral end. The central part of the nerve end was placed on the bipolar stimulating electrodes (platinum wires) and stimulated with rectangular pulses (0.1 Hz, 1 msec, supra-maximal voltage). The recording portions were right posterior sigmoid gyrus (PSG) of the cortex, right N. ventralis posterolateralis (VPL) of the thalamus (A: 9, L: 7, H: 2), right N. centralis medialis (CM) of the thalamus (A: 7, L: 3, H: 1) and right ventrolateral funiculus (VLF) of the spinal cord at the level of T3–T4. A silver ball electrode for recording the cortical evoked potentials, a stainless steel pipe insulated by enamel except for the tip (diameter of 0.5 mm) for the subcortical evoked potentials and a tungsten wire insulated except for the tip (tip diameter of 0.1 mm) for the spinal evoked potentials were used.

2) Effects on thalamo-cortical pathways: The evoked potentials were recorded from PSG of the cortex following electrical stimulation (0.3 Hz, 0.2–0.5 msec, supra-maximal voltage) of CM or VPL of the
thalamus. Bipolar concentric electrodes for stimulation and a monopolar silver ball electrode for records were used.

3) Effects on direct cortical response: Direct cortical response was recorded from the surface portion close to the stimulating electrode (within 5 mm) in PSG of the cortex following its electrical stimulation (0.1 Hz, 0.1 msec, supramaximal voltage). The electrodes made from platinum wires insulated by enamel except the tip were used for stimulation and recording.

In all experiments on the evoked potentials, monopolar recording was made with reference to an indifferent electrode in the cranium. The exposed portions of the central nervous system and peripheral nerve were covered with warm liquid paraffin. The experiments were started 3 hr after termination of ether anesthesia. Effects of drugs on the evoked potentials were determined by comparing the average of the peak to peak amplitude of each response before and after their administrations.

In above experiments, electroencephalograph (Nihon Kohden ME-95D) for recording the EEG and stimulator (Nihon Kohden SEN-3201) for the peripheral nerve and brain stimulation were used. The evoked potentials were amplified through preamplifiers (Nihon Kohden MEZ-7101, AVB-9, AVH-9) and displayed on a dual beam oscilloscope (Nihon Kohden VC-9). The responses following 10 successive stimulations were averaged by means of the averaging data processor (Nihon Kohden ATAC-201) and averaged patterns were displayed on a XY-recorder (Watanabe inst. WX-4401).

The test drug, nefopam (Riker, USA) is a white powder soluble in water and was dissolved in Ringer's solution. Other drugs used were morphine hydrochloride (Sankyo), levallorphan tartrate (Takeda), gallamine triethiodide (Teikoku Kagaku), anesthetic ether (Showa Ether), xylocaine jelly (Fujisawa) and pentobarbital sodium (Kyoritsu Shoji). Drugs were injected intravenously and the doses were given in terms of the salts.

RESULTS

I) Electroencephalogical studies

In rabbits, intravenous administration of nefopam (2.5 and 5.0 mg/kg, N=3, respectively) caused no detectable changes in the gross behavior except for tachypnea, while in cats, mydriasis, salivation, tachypnea and vomiting were frequent. The spontaneous EEG exhibited a slight alert pattern consisting of low voltage-fast waves in the sensorimotor cortex and amygdala and regular theta waves in the hippocampus in rabbits. These changes appeared quickly and lasted for about 1 hr. Further increase up to 5 mg/kg (N=3) did not enhance the EEG changes.

II) Effects of afferent pathways of the splanchnic nerve

1) Evoked potentials recorded from the cerebral cortex: The evoked potentials recorded from the posterior sigmoid gyrus of the cortex following splanchnic nerve stimulation were composed of a first biphasic negative-positive wave with a latency of 18–30 msec succeeded by a negative wave. Intravenous administration of nefopam suppressed the first negative-positive wave. Mean maximum decreases in amplitude at doses of 1.0 (N=5), 2.5 (N=5) and 5.0 mg/kg (N=5) were 47%, 46% and 56%, respectively. These suppressive effects lasted for 15 min, 90 min and more than 90 min, respectively (Fig. 2, Table 1). Morphine (5 mg/kg, i.v., N=5) also suppressed a first negative-positive wave by 58% (Table 1) and the effect lasted for over 60 min.

2) Evoked potentials recorded from VPL: The evoked potentials recorded from VPL of the thalamus following splanchnic nerve stimulation were composed of a biphasic...
negative-positive wave with a latency of 13–18 msec succeeded by a negative wave. Intravenous administration of 2.5 (N=5) and 5.0 mg/kg (N=7) of nefopam suppressed markedly the first negative-positive wave (37% and 60%, respectively). These effects lasted for more than 60 min (Fig. 2). Morphine (5.0 mg/kg, i.v., N=3) suppressed more markedly the first negative-positive wave (84%) (Table 1) and the effect lasted for over 60 min.

3) Evoked potentials recorded from CM: The evoked potentials recorded from CM of the thalamus following splanchnic nerve

Table 1. Effects of nefopam and morphine on the potentials evoked in cats from the posterior sigmoid gyrus, the nucleus ventralis posterolateralis, the nucleus centralis medialis and the ventrolateral funiculus following splanchnic nerve stimulation.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Percentage decrease in peak amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PSG</td>
</tr>
<tr>
<td>Nefopam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>47± 3(3)</td>
<td>8± 8(4)</td>
</tr>
<tr>
<td>2.5</td>
<td>46±10(5)</td>
<td>37± 9(5)</td>
</tr>
<tr>
<td>5.0</td>
<td>56± 5(5)</td>
<td>60±10(7)</td>
</tr>
<tr>
<td>Levallorphan*</td>
<td>6.0</td>
<td>72± 4(4)</td>
</tr>
<tr>
<td>Nefopam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>5.0</td>
<td>63± 6(2)</td>
</tr>
<tr>
<td>Morphine+levallorphan**</td>
<td>5.0</td>
<td>10± 1(3)</td>
</tr>
</tbody>
</table>

Values represent the mean±S.E. for the number of cats indicated in parenthesis. Each response was maximally attained within 5–30 min following administration of drugs.

*: Levallorphan (0.5 mg/kg, i.v.) was given 10 min prior to nefopam administration.

**: Levallorphan (0.5 mg/kg, i.v.) was given when depressant effects of morphine reached to the maximum.
stimulation were composed of a small early positive-negative wave followed by a large late negative wave with a latency of about 95 msec. Intravenous administration of 1.0 (N=3) and 2.5 mg/kg (N=4) of nefopam slightly depressed (20% and 28%, respectively) a large late negative wave and a more marked suppression (63%) was observed at a dose of 5.0 mg/kg (N=7). These depressant effects lasted for 15 min, 30 min and more than 60 min, respectively (Fig. 2). Intravenous administration of morphine (5.0 mg/kg, N=2) caused a marked suppression (about 100%) of a large late negative wave lasting for more than 60 min (Table 1).

4) Evoked potentials recorded from the spinal cord: The evoked potentials recorded from VLF of the spinal cord at the level of T3-T4 following splanchnic nerve stimulation had a latency of 5-7 msec and an amplitude of about 1.3 mV. The evoked potentials obtained in spinal cats and pentobarbital-anesthetized cats were stable to the external stimuli (pinching of the body with forceps or tapping of the stereotaxic apparatus) and amplitude tended to be much higher than that of intact cats.

In intact cats, the evoked potentials were suppressed by intravenous administration of nefopam. Mean maximum decreases at

![Fig. 3. Effects of nefopam (5.0 mg/kg) on evoked potential in a cat recorded from the ventrolateral funiculus of the spinal cord following splanchnic nerve stimulation in various preparations. Stimulating voltage (3 V) was provided by single square pulse (1 msec duration) at an interval of 10 sec.](image)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Intact</th>
<th>Spinal</th>
<th>Pentobarbital -Na anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nefopam</td>
<td>2.5</td>
<td>38±10(4)</td>
<td>5± 4(3)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>64± 8(6)</td>
<td>2± 4(3)</td>
<td>0± 3(3)</td>
</tr>
<tr>
<td>Morphine</td>
<td>5.0</td>
<td>56± 9(5)</td>
<td>0± 9(2)</td>
<td>—</td>
</tr>
</tbody>
</table>

Values represent the mean±S.E. for the number of cats indicated in the parenthesis. Each response was maximally attained within 5-30 min following administration of drugs.
doses of 1.0 (N=4), 2.5 (N=4) and 5.0 mg/kg (N=6) were 35%, 38% and 64%, respectively (Table 1). These effects lasted for more than 30–60 min (Fig. 3). These suppressive effects of nefopam on the splanchnic-spinal afferent pathway were never seen in spinal cats and pentobarbital-anesthetized cats (Fig. 3, Table 2). Intravenous administration of 5.0 mg/kg (N=5) of morphine suppressed the evoked potentials (56%) for more than 60 min in intact cats, but not in spinal cats (Table 2).

5) Effects of levallorphan on the depressive effects of nefopam and morphine on various evoked potentials: In the evoked potentials recorded from PSG of the cortex, CM and VPL of the thalamus and VLF of the spinal cord following splanchnic nerve stimulation, the depressant effects of morphine (5.0 mg/kg, i.v., N=5), but not of nefopam (5.0 mg/kg i.v., N=4), were antagonized by intravenous administration of 0.5 mg/kg of levallorphan (Table 1).

6) Effects on the evoked potentials recorded from the cerebral cortex by VPL stimulation: The evoked potentials recorded from the posterior sigmoid gyrus of the cortex by stimulation of VPL of the thalamus were composed of an initial triphasic wave (positive-negative-positive) with a latency of a few msec succeeded by a late positive wave with a latency of about 15 msec.

Intravenous administration of 2.5 (N=5) and 5.0 mg/kg (N=7) of nefopam slightly enhanced the amplitude of both initial triphasic and late positive waves (Fig. 4). Intravenous administration of 5.0 mg/kg (N=2) of morphine tended to enhance the late positive wave.

7) Effects on the evoked potentials recorded from the cerebral cortex by CM stimulation: The evoked potentials recorded from the posterior sigmoid gyrus of the cortex by stimulation of CM of the thalamus were composed of an early small biphasic wave (positive-negative) followed by a late large biphasic one (negative-positive).

Intravenous administration of 2.5 (N=4) and 5.0 mg/kg (N=5) of nefopam had no significant effect on either potential (Fig. 4). Intravenous administration of 5.0 mg/kg (N=2) of morphine slightly suppressed these evoked potentials.

8) Effects on the direct cortical response: The evoked potentials recorded from the surface portion close to the stimulating
electrodes (within 5 mm) following electrical stimulation of the cerebral cortex were composed of two negative components, early negative wave evoked immediately after stimulation succeeded by slow negative wave with a latency of 18–25 msec and a duration of 100–175 msec.

Intravenous administration of 2.5 (N=3) and 5.0 mg/kg (N=3) of nefopam slightly enhanced the amplitude of the slow negative component (21–39%) without any consistent effect on the fast negative wave (Fig. 5). Intravenous administration of 5.0 mg/kg (N=3) of morphine slightly suppressed both components (12–23%).

**DISCUSSION**

Nefopam induced arousal patterns in the spontaneous EEG of the rabbit. These changes in EEG patterns differed from those caused by morphine which produced drowsy patterns in rabbits but not in cats. These results suggest that nefopam has excitatory actions on the central nervous system.

In intact cats, both nefopam and morphine suppressed the evoked potentials recorded from the cerebral cortex (PSG), thalamus (CM, VPL) and spinal cord (VLF) following splanchnic nerve stimulation. The suppressive effect of nefopam (5 mg/kg, i.v.) on the evoked potentials recorded from PSG and VLF was comparable to that of morphine (5 mg/kg, i.v.). However, the observations that the evoked potentials recorded from PSG following stimulation of VPL or CM of the thalamus were either enhanced or not influenced by nefopam administration and that the direct cortical response was enhanced suggest that nefopam may have an inhibitory effect on the spinal sensory transmission.

The depression of the spinal evoked potential was not observed in spinal cats (C₁–C₂ transection) even in a relatively large dose of nefopam (5 mg/kg, i.v.). This finding indicates that the inhibitory effect of nefopam, at the doses used, is not mediated by the direct action on the spinal cord, but rather through its facilitatory action on the descending inhibitory mechanism, originating in the supra-spinal structure.

Satoh and Takagi (5) suggested that there may be, in cats, at least two descending sensory-regulating mechanisms on the splanchnic afferent pathways in the ventrolateral funiculus of the spinal cord, one susceptible to anesthetics (6) and the other resistant to them. Morphine depressed the spinal potential evoked by splanchnic nerve stimulation, even under pentobarbital anesthesia (5, 7), whereas nefopam did not suppress it under the same conditions. Therefore, nefopam may inhibit splanchnic afferent pathways in the spinal cord by reinforcing descending inhibitory systems in the supra-spinal structure, in a manner which differs from that seen with morphine.

It is worth noting that the depressant effects on splanchnic afferent pathways produced by morphine were antagonized by levallorphan, while the effects by nefopam were not influenced. These findings suggest that actions of nefopam are not related to opiate receptors in the central nervous system.

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**REFERENCES**

