THE DEVELOPMENT OF TOLERANCE TO AND OF PHYSICAL DEPENDENCE ON MORPHINE FOLLOWING INTRAVENTRICULAR INJECTION IN THE RAT

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The mechanism of the development of tolerance to and of physical dependence on morphine has been examined from a wide range of aspects. One of the approaches is to investigate the participation of brain biogenic amines in the actions of morphine. In 1954 Vogt (1) found a reduction of the content of hypothalamic norepinephrine after a subcutaneous morphine in the cat. Following her studies, some investigations were made on the change of the level of brain norepinephrine, dopamine and 5-hydroxytryptamine (5-HT) after either single or repeated administration of morphine.

Recently it was found that the analgesic action of morphine was abolished when either catecholamine releasers such as reserpine and tetrabenazine (2), or a potent inhibitor of tyrosine hydroxylase, α-methyltyrosine (3), was given several hours before morphine in mice. This fact has suggested an important role played by catecholamines in the analgesic action of morphine. This suggestion has been further supported by the observation that the abstinence symptoms manifested by nalorphine in morphinized rats is deteriorated by a pretreatment with α-methyltyrosine or disulfiram (4).

The present study deals with the development of tolerance to analgesic action of morphine and the change of brain norepinephrine content after intraventricular injection of morphine into the rat. The abstinence symptoms induced by levallorphan in the rat that received repeated intraventricular injections of morphine are also reported.

METHODS

1. Intraventricular injection

Male rats of Donryu strain weighing 230 to 300 g were used. Under ether anesthesia a steel cannulae was inserted 5 mm deep into the rat brain at a point of 3.0 mm caudal and 1.0 mm lateral from the bregma, and it was fixed by the dental cement. A cannulated rat was fed and given water ad libitum in an individual cage. Five to seven days after the cannulation drugs were injected into the cerebral ventricles through the cannulae. Drugs were dissolved in 0.9% NaCl solution brought to pH 7.0 with 0.1 N HCl or 0.1 N NaOH and injected intraventricularly in a volume of 25 μl.

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2. Measurement of analgesic activity and body temperature

Analgesic activity of morphine in the rat was measured with the apparatus of Takagi et al. (5). A rat was held on a table and the cylinder-piston with a round edge was applied to the right hind paw. An increasing pressure (10 mmHg per second) was added on the cylinder-piston until the rat showed pain reactions. The pressure to which the rat reacted was recorded on a kymograph in mmHg as pain threshold. If an animal did not react, the pressure was released at 200 mmHg. The rectal temperature was measured by a thermister-thermometer. The probe was inserted 5 cm deep into the rectum.

3. Determination of brain norepinephrine

The animals were sacrificed by decapitation two hours after the injection of morphine and the heads were frozen in liquid nitrogen. Three brains were pooled and homogenized in 15 ml of ice-cold 0.4 N perchloric acid. Norepinephrine was analyzed by the method of Bertler et al. (6). No corrections were made for the recovery of 60 to 65% of norepinephrine.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Writhe</td>
<td>3</td>
<td>Writhe and squirm on the floor of the cage</td>
</tr>
<tr>
<td>Squealing</td>
<td>2</td>
<td>Provoked by a gentle touch on the thorax</td>
</tr>
<tr>
<td>Scratching</td>
<td>1</td>
<td>Scratch the face with the fore limb</td>
</tr>
<tr>
<td>Restlessness</td>
<td>1</td>
<td>Rub the head against floor and dig a hole</td>
</tr>
<tr>
<td>Wet dog</td>
<td>1</td>
<td>Shake the whole body</td>
</tr>
<tr>
<td>Ptosis</td>
<td>1</td>
<td>Closed eyelids during movements</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1</td>
<td>Expulsion of soft and liquid faeces</td>
</tr>
</tbody>
</table>

4. Observation of the development of tolerance and physical dependence

The development of tolerance to morphine analgesia was assessed by measuring the pain thresholds before and after repeated intraventricular injections. After repeated administrations morphine was substituted by levallorphan tartrate and the precipitated abstinence symptoms were observed for one hour and scored according to the scale of Buckett (7), and Kuhn and Friebel (8), as modified by author (Table 1). The dose of the drug and the time of injection are given in the text.

**Fig. 1.** Change in pain threshold following intraventricular injection of morphine. Each point is the mean value obtained from seven rats. The bar represents the standard error of the mean.
RESULTS

1. Analgesia, hyperthermia and inhibition of spontaneous movements

Intraventricular injection of morphine hydrochloride produced a significant rise in pain threshold in a dose of 5 to 30 μg. The pain threshold was observed to rise 5 minutes after injection of morphine in a dose of 6 μg, reached maximum after 60 minutes, and recovered to preinjection level after 120 minutes (Fig. 1). The rat also showed miosis, exophthalmos, rhinorrhea, catatonic-like posture and rise in rectal temperature. Rectal temperature of the rat reached a maximum level 60 minutes after injection and recovered to preinjection level 180 to 300 minutes after injection (Table 2).

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of exp.</th>
<th>Body temperature (°C ± S.E.)</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>25 μl</td>
<td>37.6 ± 0.3</td>
<td>—</td>
</tr>
<tr>
<td>Morphine</td>
<td>3.8 μg</td>
<td>37.8 ± 0.2</td>
<td>40.7 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>7.5 μg</td>
<td>37.5 ± 0.03</td>
<td>40.6 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>15.0 μg</td>
<td>37.5 ± 0.1</td>
<td>40.6 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>30.0 μg</td>
<td>37.3 ± 0.1</td>
<td>40.3 ± 0.2*</td>
</tr>
</tbody>
</table>

* Values differ from saline control. P<0.01

The dose of 150 μg of morphine produced catatonic-like posture in the rat. They became irritable to touch and sound a few minutes after injection. Convulsive running accompanied with squealing and mydriasis was induced by external stimuli within 5 minutes of injection. After the convulsions had disappeared the spontaneous movements were depressed for 4 to 5 hours.

2. Effect on brain norepinephrine

When 7.5 μg and 30 μg of morphine hydrochloride were injected intraventricularly, brain norepinephrine was reduced by 40% and 50% of saline control, respectively, 2 hours after the injection (Fig. 2).

However, no reduction of brain norepinephrine content was observed

![Graph showing effect of intraventricular morphine on norepinephrine content of the rat brain. Rats were decapitated 2 hours after injection and three brains were pooled. Number of observations is within brackets. The vertical bar represents the standard error of the mean.](image-url)
after intraventricular injection of 30 μg of morphine in the rat that had received repeated intraventricular morphine in doses from 7.5 until 30 μg, twice daily (at 10 a.m. and 8 p.m.) for 9 days. In the control group of rats which had received intraventricularly 0.9% NaCl solution for 9 days, brain norepinephrine content was reduced by the intraventricular injection of 30 μg of morphine.

3. Development of tolerance to analgesic action of morphine

Repeated intraventricular injections of morphine, once daily for 15 days, induced the development of tolerance to analgesic effect (Fig. 3). When an initial dose of 10 μg of morphine was given, it produced a rise in pain threshold for 120 minutes and the maximum pain threshold reached to 185 mmHg. On the fifth day the maximum threshold decreased to 84 mmHg and the duration of action was shortened to 90 minutes. When the dose was increased to 30 μg on the sixth day, the maximum pain threshold increased to 175 mmHg. However, it was also decreased to 135 mmHg after the same dose was maintained for 5 days. Similar change of the response was also observed on increasing the dose to 60 μg. Thus, whenever the dose of morphine was stepwise increased, the initial injection of morphine produced a distinct rise in pain threshold and a long-lasting central depression. However, when injection of increased dose of morphine was repeated, the rise in pain threshold was rapidly diminished. Control rats given 0.9% NaCl solution did not show any significant rise in pain threshold.

Then, two groups of rats which had received repeated intraventricular injections of either morphine or 0.9% NaCl solution for 15 days were given 15 mg/kg of morphine subcutaneously and the pain threshold was measured. The first group of rats tolerant to intraventricular morphine (60 μg) showed a smaller rise in pain threshold, 142 mmHg. On the other hand, the second group which had received intraventricular 0.9% NaCl solu-
tion showed the maximum pain threshold (over 200 mmHg). The difference of the pain threshold between the two groups was statistically significant.

4. Observation of the abstinence symptoms

A group of rats was given morphine by intraventricular injection three times a day (at 8 a.m., 4 p.m. and 12 a.m.) for 9 days (Group 1). The administration of morphine hydrochloride was started with 10 μg, the dose being subsequently increased to 20 μg and then 40 μg every three days. Another group was given morphine hydrochloride in increasing dose, from 10 to 40 mg/kg, by subcutaneous injection in the same time-schedule as described above (Group 2). On the tenth day levallorphan tartrate, 50 μg i.Vent. or 2 mg/kg i.p., was administered 8 hours after the last dose of morphine.

<p>| TABLE 3. Abstinence symptoms induced by levallorphan tartrate after repeated injections of morphine for 9 days. |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Intraventricular morphine (Group 1)</th>
<th>Subcutaneous morphine (Group 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Writhing</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Squealing</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Scratching</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Restless.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Wet dog</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ptosis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total score</td>
<td>4 3 4 5 4 3 2 3 7 10 6</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: I-L : intraventricular injection of 50 μg levallorphan tartrate  
P-L : intraperitoneal injection of 2 mg/kg levallorphan tartrate

Levallophorphan produced weak abstinence symptoms (total score = 2 to 5) in rats of Group 1 whether it was given intraventricularly or intraperitoneally (Table 3). The abstinence symptoms were characteristic in scratching, restlessness, "wet dog" shake, and ptosis. More intense abstinence symptoms such as squealing, diarrhoea, and writhing, were not always observed in Group 1, but they were distinctly observed in Group 2 (total score = 6 to 10). Weight loss during one hour-observation appeared in both groups. Levallophorphan produced no abstinence symptoms in the control group of rats administered 0.9% NaCl solution.

In the preliminary experiment, intraventricular treatment with either 60 μg of norepinephrine or 40 μg of dopamine 60 minutes prior to administration of levallorphan improved the abstinence symptoms in the rat which received repeated subcutaneous injections of morphine.

DISCUSSION

Morphine hydrochloride in the doses from 5 μg upwards produced analgesic effect when injected into the cerebral ventricle of the rat. Until the beginning of the analgesic response it took a few minutes, so that it did not differ significantly from the time
taken with intraperitoneal injection. This could explain that morphine is very permeable
to the central nervous system after intraperitoneal injection. The site of action of mor-
phine in analgesia was attributed to the central gray matter surrounding the third ventricle
on the basis of observations after intracerebral injection in the rabbit (9). However,
according to the electrophysiological studies in cats, morphine has an inhibitory effect
on afferent pathways for nociceptive stimulation, on perception areas in cerebral cortex,
and on efferent pathways for pain response. Consequently morphine analgesia is probably
due to these inhibitory effects in various sites of the central nervous system.

It has been reported that morphine exerts dual action on body temperature, hyper-
thermia and hypothermia, when given by intracerebral injection as well as systemic ad-
ministration. Lotti et al. (10) suggested that hypothermia might be due to a direct effect
of morphine on the thermorgulatory centers in the anterior hypothalamus because an
injection of morphine into the anterior and/or preoptic hypothalamic nuclei of rats pro-
duced a fall in rectal temperature which directly related with the decrease in oxygen con-
sumption. In the present experiments intraventricular injection of morphine did not
produce a fall but a rise in rectal temperature of rats. Since the spontaneous move-
ments of the rats after morphine were depressed completely, the hyperthermia might be caused
by thermogenesis due not to muscular activity. The fact that a low dose of norepine-
phrine when given intraventricularly induced hyperthermia in the rat (11, 12), seems to
suggest that morphine which produced hyperthermia in the present experiments affected
indirectly through the release of norepinephrine. When a low dose of morphine was
administered intraperitoneally, a rise in rectal temperature usually preceded by a very
short-lasting fall was observed. But a fall in rectal temperature was not observed with
increasing dosage. Thus the effects of morphine administered intraventricularly in the
rat were very similar to the effects of subcutaneous morphine. The development of
tolerance was observed on both analgesic effect and depression of spontaneous movements
produced by intraventricular injection as well as subcutaneous injection of morphine.
Excitatory autonomic responses such as salivation, lacrimation and mydriasis, respiratory
depression, and clonic convulsions were in good agreement with those reported in cat, dog and man (13-15).

An attempt has been made to examine the participation of chemical transmitter
candidates such as acetylcholine, catecholamines, 5-HT and GABA, in the actions of
morphine on the central nervous system (16-18). An administration of morphine exerted
dual action on brain norepinephrine content; in certain doses it produced an increase and
in other doses it showed a decreasing effect (19). In the present experiments a significant
decrease in brain norepinephrine was found in rats after intraventricular injection of
morphine in doses of 7.5 to 30 μg. The explanation that the result reflected the change
of norepinephrine in hypothalamus could be supported by the following facts, that is,
hypothalamus was rich in norepinephrine and morphine given intraventricularly was made
contact with hypothalamus situated surrounding the third ventricle. When tolerance
had developed to analgesic action and to depression of spontaneous movements with
repeated intraventricular injections of morphine, brain norepinephrine content decreased no longer with 30 μg of morphine. In the electron-microscopic observation of granular vesicles contained in adrenergic nerve fibers of the pancreas, Graham et al. (20) presented the possibility of the disturbance of the norepinephrine storage in granular vesicles by morphine. The disappearance of the decreasing effect of morphine on brain norepinephrine after repeated injections seems to be explained by the fact that the synthesis of norepinephrine was accelerated during chronic administration of morphine (21). Takagi and Kuriki (22) found in mice that the development of tolerance to morphine analgesia was markedly suppressed when tetrabenazine was given prior to daily administration of morphine. On the other hand, in mice which received p-chlorphenylalanine before and after repeated morphine injections, tolerance to morphine and naloxone-induced abstinence symptoms were reduced markedly (23). These facts suggest that biogenic amines are involved in the development of tolerance to morphine.

Isbel and Fraser (24) have described the abstinence symptoms disclosed by the injection of nalorphine in morphine addicts. Similar phenomena were observed in animals chronically administered with morphine (25, 26). Some abstinence symptoms manifested by levallorphan were observed in the rat which had received repeated intraventricular injections of morphine. They were characterized by "wet dog" shake, restlessness and scratching. Diarrhoea was often observed in the intraventricular group (Group 1) as well as in the subcutaneous group (Group 2) when effects of morphine had disappeared before next injection. But it was rarely observed in Group 1, even when abstinence symptoms were induced by intraventricular injections of levallorphan. Typical intense abstinence symptoms, such as squealing and writhing, were not observed in Group 1, though they were often observed in Group 2. This result indicates that physical dependence produced by repeated intraventricular injections of morphine is rather moderate than that produced by subcutaneous injections. The difference of the intensity of abstinence symptoms between Group 1 and Group 2 is probably explained by a supposition that physical dependence on morphine develops only in neurons which locates surrounding cerebral ventricles following intraventricular injections and peripheral component of the abstinence symptoms is not involved in intraventricular morphine (Group 1).

The intensity of abstinence symptoms induced by nalorphine has correlation with the degree of reduction of brain norepinephrine in the dog, but there was no decrease of brain norepinephrine by nalorphine in morphinized rats (19). This fact may be caused by the differences in the rate of resynthesis of catecholamines in dogs and rats, for discharge of norepinephrine was increased and brain content of norepinephrine was decreased by nalorphine in rats treated with chronic morphine, provided that α-methyltyrosine was given prior to administration of nalorphine (26). An intraventricular pretreatment with either dopamine or norepinephrine was preliminary found to ameliorate the abstinence symptoms induced by levallorphan in subcutaneously morphine addicted rats. The effect of the catecholamines is less readily explained. High doses of the catecholamines caused sedation and a fall in body temperature. This could not exclude the possibility that the
catecholamines did not act through the restoration of norepinephrine released by induction of the abstinence symptoms, but through the nonspecific depression of the central nervous system. However, Mattila et al. (4) have obtained the deterioration of the abstinence symptoms manifested by nalorphine when brain norepinephrine had been reduced by a pretreatment with disulfiram and α-methyltyrosine in morphinized rats. These findings seem to suggest that brain norepinephrine has an important role to recover the morphine antagonist-induced breakdown of the adaptation to morphine established by chronic administration of morphine in the central neurons.

**SUMMARY**

Intraventricular injection of morphine hydrochloride in the doses from 5 μg upwards produced analgesia, hyperthermia, inhibition of spontaneous movements, autonomic excitation, and reduction of brain norepinephrine in the rat. When the administration was repeated three times a day for 9 days, the development of tolerance to morphine analgesia was observed and the decrease of brain norepinephrine was disappeared. Levallorphan tartrate induced abstinence symptoms after repeated intraventricular injections of morphine. In the preliminary experiment intraventricular pretreatment of either dopamine or norepinephrine ameliorated the abstinence symptoms induced by levallorphan. It may be suggested that brain norepinephrine has an important role to recover the antagonist-induced abstinence symptoms in animals after chronic administration of morphine.

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**REFERENCES**