The authors have reported in the previous papers that tetrabenazine antagonizes morphine analgesia in mice (1) and that it has depressive effects on some afferent pathways in the central nervous system in rabbits and cats, like those of morphine (2).

The questions arise whether the antagonistic effect by tetrabenazine of the central effects of morphine can be recognized in other species besides mice, whether all of the depressive actions of morphine on the afferent pathways of the central nervous system are suppressed by the tetrabenazine pretreatment, and whether tetrabenazine may owe its antagonistic action against morphine to its ability to deplete the catecholamines and serotonin levels in the brain.

The purpose of this study was to investigate these questions using the electro-physiological techniques.

METHODS

A total of 48 male albino rabbits weighing from 2.5 to 3.0 kg were employed in the EEG experiments and 15 adult male cats weighing from 2.5 to 3.0 kg were used for the experiments of evoked potentials.

The methods of both experiments were exactly similar to those described in the previous paper (2).

The drugs used in the present experiments were tetrabenazine methanesulphonate, morphine hydrochloride and dl-3,4-dihydroxyphenylalanine (DOPA), all of which were administered by the intravenous route.

RESULTS

1. The spontaneous EEG of unanesthetized and non-curarized rabbits restrained stereotaxically

The spontaneous EEG of unanesthetized rabbits restrained stereotaxically was classified into three patterns which were fully described in the previous paper (2). The outline of these patterns are as follows:
a) **Alert pattern**: this pattern consists of low voltage (20-50 $\mu$V), and fast waves (20–30 cps) in the motor cortex, and consists of high voltage (100-200 $\mu$V) and relatively regular slow waves (4-6 cps) in the hippocampus.

b) **Resting pattern**: high voltage, slow frequency waves (50-200 $\mu$V, 1-3 cps) accompanying spindle bursts (100-200 $\mu$V, 8-14 cps) in the motor cortex and high voltage, irregular slow frequency waves (200-400 $\mu$V, 1-3 cps) in the hippocampus.

c) **Mixed pattern**: the alert pattern and the resting pattern appear alternatively at the interval of several seconds to several minutes.

2. **Effects of tetrabenazine**

The results obtained in the present experiment was approximately same as those which have been described in the previous paper (2).

The administration of 40 mg/kg of tetrabenazine induced the characteristic slow waves in the spontaneous EEG, which lasted about 30 minutes. These waves gradually shifted to the usual resting pattern on about 30 minutes after the administration and after 5 hours the EEG still showed the resting pattern.

In some cases, however, the characteristic slow waves were followed by the alert pattern which lasted for 15 to 30 minutes and then the resting pattern appeared and lasted for several hours. In a dose of 20 mg/kg, the drug did not produce the characteristic slow waves and only showed the resting pattern for 2 or 3 hours after the administration. Five to 10 mg/kg of tetrabenazine caused no significant change in the EEG.

The EEG arousal response which was elicited by the stimulation of the midbrain reticular formation at 300 cps was suppressed by 40 mg/kg of the drug. The threshold voltage of stimuli of the midbrain reticular formation showed an increase of about 80 to 100 per cent of its original value at 2 or 3 hours after the injection.

The recruiting response was not changed significantly by the administration of 40 mg/kg of tetrabenazine, however, the augmenting response was depressed by the injection of tetrabenazine (40 mg/kg). The threshold voltage of the stimulation increased about 40 to 60 per cent compared with the voltage before the administration of tetrabenazine, and this was observed within 2 to 3 hours after drug injection.

The second component of the local cortical potential and the cortical potential evoked by afferent stimulation of the splanchnic nerve were suppressed by tetrabenazine (40 mg/kg). These inhibitory actions lasted for about 2 hours.

3. **Effects of morphine**

Immediately after administration of 5 mg/kg of morphine, the EEG activity of the motor cortex and the hippocampus changed into high voltage (200-300 $\mu$V) and slow waves (1-5 cps) and these waves lasted for about 15-30 minutes. Then these waves were substituted for the slow pattern with frequent appearances of the spindle bursts in the motor cortex, associated with the irregular slow waves in the hippocampus. The same type of waves as in the pre-administration stage began to reappear 2 to 3 hours after
FIG. 1. Effect of morphine (5 mg/kg) on the spontaneous EEG in a normal rabbit and a tetrabenazine-pretreated rabbit.
(Left): Morphine alone.
(Right): Morphine with the pretreatment of tetrabenazine (40 mg/kg).
MC: Motor cortex, HPC: Hippocampus.
These abbreviations are also used in the following figures.

FIG. 2. Effect of morphine (5 mg/kg) on the reticular arousal response in a normal rabbit and in a tetrabenazine-pretreated rabbit.
(Left): Morphine alone.
(Right): Morphine with the pretreatment of tetrabenazine (40 mg/kg).
drug administration (Fig. 1). In a dose of 3 mg/kg of morphine, the change of EEG was slight in intensity and short in duration, and returned to a control pattern one hour after the administration.

In order to test the effect of drugs on the reticular arousal response and on the augmenting response, electrical stimuli were given every 15 minutes for the first one hour following the injection and thereafter stimuli were given every 30 minutes.

Though the spontaneous EEG changed into the slight slow pattern after administration of 3 mg/kg of morphine, the reticular arousal response was hardly affected. The dose of 5 mg/kg of morphine depressed this response and the threshold voltage increased about 60 per cent from 15 minutes to one hour after drug injection. The intensity of the depression to this response began to decline from about 1.5 hours after administration of morphine (Figs. 2 and 7).

The recruiting response was facilitated by the injection of morphine (5 mg/kg) and the threshold voltage showed a decrease of 30 or 40 per cent of the original value. Two hours after the administration, the threshold voltage returned to the control level (Figs. 3 and 7).

The augmenting response was suppressed by the administration of 5 mg/kg of morphine, and the increase of 40 to 60 per cent of the threshold voltage was observed one hour after drug administration and lasted for about 2 hours (Figs. 4 and 7).

Fig. 3. Effect of morphine (5 mg/kg) on the recruiting response in a normal rabbit and in a tetrabenazine-pretreated rabbit.
(left) : Morphine alone.
(right) : Morphine with the pretreatment of tetrabenazine (40 mg/kg).
Fig. 4. Effect of morphine (5 mg/kg) on the augmenting response in a normal rabbit and in a tetrabenazine-pretreated rabbit.
(Left) : Morphine alone.
(Right) : Morphine with the pretreatment of tetrabenazine (40 mg/kg).
SC : Sensory cortex.

Fig. 5. Effect of morphine (5 mg/kg) on the local cortical potential by single shock of the surface of the gyrus lateralis in a normal cat and in a tetrabenazine-pretreated cat.
(Left) : Morphine alone.
(Right) : Morphine with the pretreatment of tetrabenazine (40 mg/kg).
The second component of the local cortical potential and of the potential evoked by stimulation of splanchnic nerve in pentobarbital anesthetized cats were markedly depressed by the administration of 6 mg/kg of morphine (Figs. 5 and 6). Fifteen minutes after the injection, both potentials completely disappeared and did not reappear even though following the application of 2 or 3 times stronger voltage than that in the control stage. The inhibitory action of morphine to these responses continued for one to 2 hours.

4. Effects of morphine on the tetrabenazine-pretreated animals

The maximal effect of tetrabenazine on the spontaneous EEG and the reticular arousal response was obtained within 1.5 hours after the administration and lasted for several hours. Therefore, certainly depressive dose, 5 mg/kg of morphine, was administered 3 hours after the administration of tetrabenazine and the influences of the tetrabenazine pretreatment on the central actions of morphine were investigated.

It was difficult to evaluate the effect of morphine on the spontaneous EEG in the tetrabenazine-treated rabbits, as their background EEG activities has already shown the resting pattern after tetrabenazine administration. However, the characteristic slow waves observed following the administration of morphine alone, were never obtained in the case of the pretreatment with tetrabenazine (Fig. 1).

In the tetrabenazine-pretreated rabbits the elevation of the threshold voltage of the reticular arousal response was only 15 to 30 per cent within 30 minutes after injection of morphine and returned to the control level one hour after injection (Figs. 2 and 7).
ANTAGONISM OF MORPHINE BY TETRABENAZINE

FIG. 7. Changes in the threshold voltage of the reticular arousal response, the recruiting response and the augmenting response after the administration of morphine (5 mg/kg).
(Left) : Morphine alone.
(Right) : Morphine with the pretreatment of tetrabenazine (40 mg/kg).
Abscissa : Time.
Ordinate : Change in the threshold voltage (%).

The facilitatory effect of morphine on the recruiting response was markedly suppressed by the pretreatment with tetrabenazine (Figs. 3 and 7).

Morphine (5 mg/kg) suppressed the augmenting response and the threshold voltage increased by 40 to 60 per cent when it was administered alone, while in the case of the pretreatment with tetrabenazine the suppressive effect of morphine on this response was not observed any longer (Figs. 4 and 7).

The dose of 6 mg/kg of morphine which depressed the local cortical potential in cats was administered at 1.5 hours after injection of tetrabenazine. The inhibitory effect of morphine on this response was not apparent in tetrabenazine pre-administered cats (Fig. 5).

The suppressive effect of morphine on the potential evoked by stimulation of the splanchnic nerve was not observed in the case of the pretreatment with tetrabenazine (Fig. 6). In this case, the amounts of morphine necessary to block both potentials increased at least 2 or 3 times as much as those of non-treated animals.

FIG. 8. Effect of morphine (5 mg/kg) after tetrabenazine- and DOPA-pretreatment on the spontaneous EEG.
5. Effects of morphine on the animals pre-treated with both tetrabenazine and DOPA

When DOPA (40 mg/kg) was given into rabbits which had received 40 mg/kg of tetrabenazine 3 hours earlier, the resting or the mixed pattern induced by tetrabenazine was changed into the alert pattern and this pattern lasted for more than 30 minutes. When 5 mg/kg of morphine was injected 15 minutes after administration of DOPA, a typical slow pattern appeared and an increase in the threshold voltage of the reticular arousal response was almost the same as observed after injection of morphine alone (Figs. 8 and 9). The duration of the depressive action of morphine on the response was rather longer than that of morphine alone and lasted for more than 2 hours.

DISCUSSION

When morphine was injected into rabbits or cats 3 hours after administration of tetrabenazine, the inhibitory effects of morphine on the activity of the central nervous system such as the spontaneous EEG, the reticular arousal response, the recruiting response, the augmenting response, the local cortical potential and the potential evoked by stimulation of splanchnic nerve were considerably weakened.

Takagi et al. (1) have reported that morphine analgesia in mice is antagonized by tetrabenazine when morphine is injected after administration of tetrabenazine and that the maximum antagonism is observed when tetrabenazine is injected 2-4 hours before morphine injection.

Many workers (6-9) had already observed that reserpine antagonized morphine analgesia in mice. Takagi et al. (1) also confirmed that the antagonistic phenomenon between both drugs in mice was not seen when both drugs were administered simultaneously or when reserpine was administered after morphine injection.

It was suggested that the cause of reserpine antagonism of morphine analgesia in mice is related to the release of catecholamines and/or serotonin in brain by reserpine (1, 6-9).

Pletcher et al. (3), Quinn et al. (4) and Pletcher (5) reported that single injection of tetrabenazine into rabbit caused a rapid reduction of catecholamines and serotonin contents in brain and its maximal effect is obtained in 3 to 5 hours.
ANTAGONISM OF MORPHINE BY TETRABENAZINE

Considering the above suggestions, it is probable that the antagonistic effects of tetrabenazine on the central action of morphine is related to its depleting effect of the amines. This view appears to be supported by the present experimental results; when 40 mg/kg of DOPA, the precursor of dopamine and noradrenaline, was administered 3 hours after tetrabenazine injection and then 5 mg/kg of morphine was injected 30 minutes after the administration of DOPA, tetrabenazine did not show any antagonism to the central actions of morphine, and the suppressive potency of morphine was approximately the same as observed in that of morphine alone.

Takagi et al. (1) found that the antagonistic effect of tetrabenazine to morphine analgesia in mice was considerably weakened by the administration of DOPA but not by that of 5-HTP. Carlsson et al. (10) also observed that the sedative action of reserpine was returned to the control state by DOPA administration but not by 5-HTP.

These suggest that catecholamines such as noradrenaline and dopamine might play an important role in the antagonism by tetrabenazine of the central depressant effect of morphine. Further study on the changes of the contents of catecholamines in the brain after administration of tetrabenazine, morphine, DOPA, alone and in combination, is in progress.

The authors have recently ascertained that the sites of action of tetrabenazine are similar to those of morphine except their effects on the recruiting response (2).

In this connection, it is interesting to note that nalorphine has the same site and mode of actions on the afferent pathways of the central nervous system (11) as those of morphine reported by Fujita et al. (12, 13) and Takagi et al. (14, 15) and that nalorphine shows the antagonism in every site of depressive action of morphine (11) like that of tetrabenazine (Table 1).

TABLE 1. The sites and modes of actions of morphine, nalorphine, tetrabenazine on the central nervous system.

| EEG and evoked potential | Morphine 5-8 mg/kg | Nalorphine 20 mg/kg | Tetrabenazine 40 mg/kg | Antagonism Morph. + Nalorph. | Tetra. + Morph.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous EEG R.</td>
<td>slow variable</td>
<td>fast</td>
<td>slow</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EEG arousal response C.</td>
<td>(↓)</td>
<td>↑</td>
<td>↓</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Recruiting response R.</td>
<td>↑</td>
<td>↓</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Augmenting response C.</td>
<td>↓</td>
<td>↓</td>
<td>(0)*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Local cortical response C.</td>
<td>↓</td>
<td>0</td>
<td>↓</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Splanchnic N. →Cortex C.</td>
<td>↓</td>
<td>↓</td>
<td>(0)*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spinal reflex</td>
<td>↓</td>
<td>0</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

R.: Rabbit ↑: Inhibition +: Antagonistic
C.: Cat ↑: Facilitation 0: No effect
* Effect of 10 mg/kg of nalorphine. ?: Not investigated
These findings raise the question that tetrabenazine, besides having an antagonistic action on morphine, exerts an analgesic action of its own, like that of nalorphine. This problem remains to be investigated through the clinical investigation.

**SUMMARY**

1. Tetrabenazine in a dose of 40 mg/kg produced the characteristic slow waves in the spontaneous EEG of the rabbit. Both the reticular arousal response and augmenting response in rabbits were depressed by the drug, but the recruiting response in them was not changed significantly. The second component of the local cortical potential and the cerebral potential evoked by afferent stimulation of the splanchnic nerve in cats were also suppressed by 40 mg/kg of tetrabenazine.

2. The dose of 5 mg/kg of morphine induced high voltage, slow waves (200-300 μV, 1-5 cps) in the motor cortex of the rabbit. The effect of morphine on the spontaneous EEG continued for 2 to 2.5 hours. Morphine in a dose of 5 mg/kg depressed the reticular arousal response and the augmenting response in rabbits and on the contrary, the recruiting response in rabbits was facilitated in the same dose of morphine. The second component of the local cortical potential and of the potential evoked by stimulation of the splanchnic nerve in cats were markedly suppressed by 6 mg/kg of morphine.

3. The characteristic slow waves which was observed after injection of morphine alone was not observed in the tetrabenazine-pretreated rabbits. The suppressive response and the augmenting response was markedly suppressed by the pretreatment with tetrabenazine. The facilitatory effect of morphine on the recruiting response was markedly hindered by the pretreatment with tetrabenazine. The inhibitory effects of morphine on the local cortical potential and the potential evoked by stimulation of the splanchnic nerve were not apparent when tetrabenazine was pre-administered.

4. The resting or the mixed pattern induced by tetrabenazine was changed into the alert pattern after DOPA administration in a dose of 40 mg/kg and this pattern lasted for more than 30 minutes.

5. The antagonism of the effect of morphine on the reticular arousal response by tetrabenazine was suppressed by DOPA administration when it was administered 3 hours after tetrabenazine.

6. The antagonism between tetrabenazine and morphine is recognized through the electrophysiological investigation and the mechanisms of this antagonism are discussed in relation to the depleting effect by tetrabenazine of catecholamines and serotonin.

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