NEUROPHARMACOLOGICAL STUDIES OF EFFECT OF NEW CENTRAL DEPRESSANT, 8-CHLORO-6-PHENYL-4H-s-TRIAZOLO [4,3-a] [1,4] BENZODIAZEPINE (D-40TA) ON EEG AND CENTRAL SYMPATHETIC ACTIVATING MECHANISM IN CATS

Naohisa FUKUDA, Yoshiaki SAJI and Yuji NAGAWA

Biological Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Higashiyodogawa-ku, Osaka, Japan

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Abstract—The present study regarding the effects of 8-chloro-6-phenyl-4H-s-triazolo [4, 3-a] [1, 4] benzodiazepine (D-40TA) on the EEG arousal and sympathetic excitatory responses evoked spontaneously or by stimulation of the midbrain reticular formation, posterior hypothalamus, thalamus and sciatic nerve in the curarized, intact brain or cerveau isolé cats provided the following conclusion: D-40TA reduced the central sympathetic excitability by depressing the hypothalamus and its related structures, and limited the persistence of the EEG arousal by selective depression of the hypothalamic and probably midbrain mechanism relevant to a "tonic" EEG component with much less influence upon a "phasic" EEG component related to waking ability itself. These dual effects may be responsible for hypnogenic action of D-40TA.

A newly synthesized compound, 8-chloro-6-phenyl-4H-s-triazolo [4, 3-a] [1, 4] benzodiazepine (D-40TA) (1) has been previously found by our research group (2, 3) to be highly active in sedative-hypnotic, taming, anticonvulsive and muscle relaxant effects in various animal species. Under undisturbed conditions, especially in monkeys, an appropriate dose of this compound readily induces the sleeping state which is comparatively easily interrupted by external stimulation but is regained shortly after termination of the stimulus. Moreover, even the sublethal dose never produces an anesthetic state in rodents. In this respect, the hypnotic action of this compound appeared different from sedative or anesthetic effect of classical hypnotics such as barbiturates.

In the field of sleep physiology, it is still controversial whether or not sleep is a passive or active phenomenon: dampening of an active function of the waking center such as midbrain diencephalic activating system (4, 5) on the one hand or an active function of the hypnogenic center (6, 7) on the other. The cortical EEG arousal induced by midbrain or sensory stimulation has been claimed to consist of an initial, short-lasting "phasic" component and a delayed, prolonged "tonic" component, the latter of which is usually accompanied by motor and autonomic changes (8-10) and depends largely on the integrity of the hypothalamus (9, 11).

On such a physiological basis, a neuropharmacological approach was undertaken...
to clarify the mechanism of sedative-hypnotic action of D-40TA, by recording the responses of preganglionic cervical sympathetic discharges and EEGs to hypothalamic, thalamic, midbrain and peripheral stimulations. Diazepam and nitrazepam, the latter of which has been clinically used as a sleep-inducing agent (12–14), were also tested herein, as referential agents.

MATERIALS AND METHODS

A total of 64 cats of both sexes weighing 2.5–3.5 kg were used. Forty-four animals were used for the intact brain preparations and the rest for the cerveau isolés.

Surgical procedures: Following a tracheal intubation under ether anesthesia, the head of the animal was fixed in an ordinary position on a Todai-Noken type of stereotaxic instrument. After exposure by scalp incision, small burr holes were made in several places in the skull for insertion of the electrodes. The stereotaxic frame was then rotated about 45° to one side, and a unilateral cervical sympathetic nerve and a phrenic nerve diverging from 5th cervical cord were dissected free through a lateral neck incision.

For preparation of acute cerveau isolé, the brain-stem was transected with a dull spatula at midcollicular-prepontine level after the cerebellum had been carefully removed by aspiration.

For EEG recording and stimulation, two silver ball electrodes were placed 5 mm apart on the suprasylvian gyrus, and subcortical electrodes were implanted into the following coordinates according to the atlas of Jasper and Ajimone-Marsan (15): dorsal hippocampus (Hip), frontal (F) 2, lateral (L) 8, horizontal (H) 6; dorsomedial nuclei of the thalamus (TH), F: 8, L: 1.5, H: 3; posterior hypothalamus (PH), F: 10, L: 1–1.5, H: −4 to −5; midbrain reticular formation (MRF), F: 2, L: 4, H: −2. The subcortical electrode was made up by twisting a pair of wires insulated along their entire length except at the 0.5 mm tips, 0.3 mm in diameter and 0.5 mm in interpolar distance.

The phrenic nerve (PN) was cut as peripherally as possible, as was the preganglionic sympathetic nerve (SN) cut as close as possible to a superior cervical ganglion. These central ends were split into fine filaments which were draped over two bipolar platinum electrodes in a mineral-oil pool at 36–37°C for recording electrical nerve activities. In several experiments, a unilateral sciatic nerve was also exposed for afferent stimulation.

A polyethylene cannula was inserted into a femoral artery for blood pressure (BP) recording via a Nihon-Kohden MP-24T pressure transducer.

After all surgical procedures, systemic anesthesia was discontinued, and all wound edges and pressure points were locally anesthetized by careful infiltration of 1% carbocaine. The animals were maintained on an i.m. injection of 1 mg/kg of d-tubocurarine and additional doses as required, and were artificially respired at the rate of 25–30 strokes/min from a Palmer-type respirator. The preparation was allowed at least 3 hr for recovery from ether anesthesia before the experiment was started.

After the end of experiment, the brain was perfused by 10% formalin for histological preparation to verify the electrode placements and the transection site.
Recording and Stimulation procedures: All recordings of EEG, electrical nerve activities and blood pressure were made on a Nihon-Kohden model RM-150 polygraph. Movement of nictitating membrane (NM) on the unoperated, innervated side was simultaneously recorded by means of a Nihon-Kohden SB-1T force-displacement transducer. Stimulation of PH, MRF, TH and sciatic nerve was carried out with rectangular pulses, using a Nihon-Kohden MSE-3 electronic stimulator, of the following parameters: frequency, 100 Hz; pulse duration, 1 msec; stimuli duration, 5 sec. The stimulus intensity was raised up stepwise by 0.25 or 0.5 volts from an arbitrary intensity, usually 0.25 volts, at 5-min intervals until the thresholds to evoke the cortical EEG desynchronization (arousal response) and the apparently enhanced efferent SN discharges were obtained respectively. Simultaneously, the above stimuli at an intensity double the threshold voltage which was determined prior to injection of either the test compound or vehicle were also applied during the post-injection period to measure the duration of the EEG arousal response and the heights of the contraction of NM as well as of the BP pressor response. Frequency of the spontaneous sympathetic excitatory episodes during unstimulated periods was also computed. In several experiments, an integrator with time constant of 0.2 sec was employed for evaluating the sympathetic nerve activity. After the control values of these measurements were obtained, all measurements were made once each within 30 and 60 or 90 min following injection of either the test compound or vehicle.

Data analysis: All post-injection values are expressed as the ratios to their own pre-injection values in each preparation, these ratios being summarized as the mean and standard error (S.E.) by statistical analysis in several or more experiments. Differences between the mean ratio values obtained in each of the post-drug period and in the corresponding control period with vehicle were statistically analyzed by a student "t" test.

Drugs used: The following agents were utilized; D-40TA, diazepam, nitrazepam, d-tubocurarine (Amerizol®, Yoshitomi) and carbocaine (Carbocain®, Yoshitomi). Twenty % solution of glycofurol was used as a vehicle of the former three agents. The control experiments were made with an equal volume of this vehicle. All the agents were injected into a radial vein of the forearm via a polyethylene cannula.

RESULTS

1. Effects in intact brain preparations

1. Spontaneous EEG pattern and sympathetic activation

In undisturbed state of the curarized, intact brain cats, the neocortical EEG showed alternate occurrences of the relatively short-lasting synchronized pattern with spindle bursts and the prolonged desynchronized pattern which was accompanied by synchronized theta (4-6 Hz) waves or desynchronized (13-16 Hz) low voltage waves in the hippocampus. The latter pattern is briefly referred to as EEG arousal in this paper. Many of these spontaneous EEG arousals, especially when intensive and long-lasting, were associated with delayed sympathetic excitations, i.e., apparently and consistently increased efferent discharges of SN, contraction of NM, rise in BP and increase in PN volleys (Fig. 1). An
excitatory phase of SN activity was sometimes immediately followed by an inhibitory phase characterized by an almost disappearance of the discharges. During non-excitatory periods, SN activity consisted of either rhythmic firings synchronizing with PN volleys or continuous firings of low voltage (Fig. 1).

The spontaneous SN excitatory episodes during the pre-drug 30-min period occurred 5 times on an average and even 20 times in some cases. An i.v. injection of 20% glycofurol used as a vehicle tended to increase the frequency of the spontaneous SN excitatory episodes but did not change either the substantial EEG pattern or the BP level. D-40TA in 0.25 mg/kg markedly diminished the occurrence of the spontaneous SN excitations without any recognizable alteration in EEG leads. A higher dose, 0.5 mg/kg, as in the

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TABLE 1. Effects of D-40TA, diazepam and nitrazepam on spontaneous sympathetic excitatory episodes during the resting state in curarized cats with intact brain

| Test agents | Dose mg kg i.v. | No. of cats | Frequency ratio* (mean±S.E.) of spontaneous sympathetic excitatory episodes | 1st 0.5 hr | 2nd 0.5 hr |
|-------------|----------------|-------------|--------------------------------------------------------------------------------|--------|----------|
| Vehicle     | (Control)      | 11          | 1.63±1.54                                                                      | 1.61±0.82 |          |
| D-40TA      | 0.25           | 4           | 0.05±0.11**                                                                     | 0.10±0.11** |          |
|             | 0.5            | 7           | 0.00**                                                                         | 0.10±0.02** |          |
| Diazepam    | 4              | 3           | 0.69±0.11**                                                                     | 0.61±0.21** |          |
| Nitrazepam  | 2              | 4           | 0.00**                                                                         | 0.00**    |          |

Frequency in 0.5 hr period after test agent
Frequency in 0.5 hr period before test agent
**Statistically significant from the control values at p<0.05 level.
TABLE 2. Effects of D-40TA and nitrazepam on the threshold and duration of the EEG arousal responses induced by stimulation of the midbrain reticular formation (MRF), posterior hypothalamus (PH) or sciatic nerve in curarized cats with intact brain

| Test agents | Dose mg/kg i.v. | MRF stimulation | PH stimulation | Sciatic stimulation |
|-------------|----------------|----------------|----------------|--------------------|
|             |                | Threshold      | Duration       | Threshold          | Duration       | Threshold | Duration       |
|             |                | 1st 0.5 hr     | 2nd 0.5 hr     | 1st 0.5 hr        | 2nd 0.5 hr     | 1st 0.5 hr | 2nd 0.5 hr     |
| Vehicle     | (Control)      | ±0.00 ±0.00    | ±0.20 ±0.22    | ±0.00 ±0.00       | ±0.25 ±0.75   | ±0.00 ±0.00 | ±0.30 ±0.40   |
|             |                | (5)            | (5)            | (5)               | (4)            | (5)        | (5)            |
| D-40TA      | 0.5            | ±0.14 ±0.20    | ±0.14 ±0.08    | ±0.34 ±0.37       | ±0.17 ±0.06   | ±0.00 ±0.00 | ±0.13 ±0.03   |
|             |                | (7)            | (7)            | (7)               | (7)            | (7)        | (7)            |
| Nitrazepam  | 2              | ±0.10 ±0.12    | ±0.09 ±0.08    | ±0.10 ±0.05       | ±0.13 ±0.06   | ±0.20 ±0.11 | ±0.17 ±0.02   |
|             |                | (5)            | (4)            | (4)               | (4)            | (4)        | (4)            |
|             | 2              | ±0.20 ±0.32    | ±0.33 ±0.38    | ±0.38 ±0.47       | ±0.26 ±0.09   | —           | —              |
|             |                | (4)            | (4)            | (4)               | (3)            | (3)        | (3)            |

All data show mean ± standard error. Numerals in parentheses indicate number of cats used.

* Statistically significant from the control values at p<0.05 level.
case of 2 mg/kg of nitrazepam, abolished almost completely the autonomic excitations over 1 hr (Fig. 1). Diazepam, 4 mg/kg, was less effective. All data are summarized in Table 1.

For more than 1 hr from 1 or 2 min following an i.v. injection of D-40TA in 0.5 or 1 mg/kg, the spontaneous EEG patterns changed as follows: increase of slow (1-2 Hz) high voltage waves sometimes mixed with spindle bursts in the neocortical EEG of suprasylvian gyrus; appearance of irregular low voltage waves and intermittent slow (2 Hz) high voltage waves in association with decrease of normal theta components in the hippocampus (Fig. 1). BP level was lowered mildly for only 1 or 2 min immediately after these doses.

2. EEG arousal responses to brain and sciatic nerve stimulation

Table 2 represents the data averaged from 3–7 experiments regarding the effects of D-40TA and nitrazepam on the EEG arousal threshold of MRF, PH and sciatic nerve stimulations and on the duration when stimulated at a double intensity of the pre-injection threshold. A representative example is shown in Fig. 2.

![Fig. 2. Effect of D-40TA on EEG arousal and sympathetic excitatory responses to stimulation of the posterior hypothalamus in curarized cat with intact brain. Abbreviations are as in Fig. 1.](image-url)
Injection of 20% glycofurol used as a vehicle caused no change in the thresholds of EEG arousal responses to any stimulations but tended to prolong the arousal durations.

D-40TA in 0.5 and 2 mg/kg, and nitrazepam in 2 mg/kg tended to elevate, though insignificantly, the threshold of the EEG arousal response to MRF stimulation. Also, a significant elevation of the EEG arousal threshold in sciatic stimulation was not obtained with administration of D-40TA. It was found however, that the EEG arousal threshold in PH stimulation was significantly increased 1 hr after 0.5 mg/kg, and 0.5 as well as 1 hr after 2 mg/kg of D-40TA. Nitrazepam in the dose used here tended to show a similar effect but not significantly. The prominent effect of both compounds was a remarkable reduction in the EEG arousal duration in any stimulation. Actually, there were some cases in which the evoked EEG arousal response after administration of these compounds did not outlast the stimulus.

3. Sympathetic nerve (SN) and peripheral excitatory responses to brain and sciatic nerve stimulations

The data averaged from 4-7 experiments regarding the effects of D-40TA and nitrazepam on the threshold of SN efferent discharges augmented by brain and sciatic stimulation and on the contraction of NM as well as vasopressor response obtained by stimulation at a double intensity of the pre-injection SN threshold are outlined in Table 3. Twenty % glycofurol did not cause significant change in the SN threshold and NM response to any stimulation, but suppressed considerably the vasopressor response to MRF stimulation.

D-40TA at a low dose of 0.5 mg/kg significantly increased the threshold of SN exci-
tatory efferent discharges by PH stimulation and consequently suppressed the NM contraction without significant effect on the vasopressor response. A higher dose, 2 mg/kg, like the same dose of nitrazepam, elevated all the thresholds and depressed all peripheral responses to any stimulation except for no significant change with D-40TA in the vasopressor response to MRF stimulation (Tab. 3, Fig. 2).

The frequency and amplitude of spontaneous phrenic nerve (PN) volleys were somewhat diminished by 2 mg/kg of D-40TA. Prior to drug administration, the PN activity changed by brain and sciatic stimulations in various ways as follows: continuous firings or increased volleys during stimulation, both of which were usually followed by a brief silence and increased or prolonged volleys especially when the stimulus was intensive. The threshold of these changes in PN activity was raised up by D-40TA and nitrazepam in association with increased threshold of the EEG arousal response, especially SN discharges (Fig. 2).

II. Effects in cerveau isolé preparations

In order to further delimit the site of the depressant action of D-40TA on the EEG arousal and central sympathetic excitatory mechanism observed in the intact brain cats, a similar study was undertaken in the cerveau isolé cats, in which the main nervous connection between the brain-stem and diencephalon was cut.

1. EEG arousal response to thalamic (TH) and hypothalamic (PH) stimulations

Spontaneous EEG pattern observed after transection of the brain-stem was composed of mostly slow, high voltage waves with spindle burst in the neocortex (AS: anterior sigmoid gyrus) and slightly irregular slow (2-3 Hz) waves in the hippocampus. Following an i.v. injection of D-40TA in 0.5 mg/kg, the spontaneous neocortical EEG pattern was not substantially altered but the hippocampal activity was lowered considerably, in fact almost flattened.

In these cerveau isolés, the dorsomedial nuclei of the thalamus (TH) and the posterior hypothalamus (PH) were stimulated at high frequency before as well as after administration of D-40TA until the threshold of the EEG arousal response was obtained. Before drug administration, the most usual EEG arousal patterns consisted of fast, low voltage or almost flattened waves in the neocortex which were associated with appearance of normal theta waves or fast, low voltage waves in the hippocampus. The EEG arousal response evoked by PH stimulation was generally longer-lasting than that by TH stimulation (Figs. 3, 4).

Since the background EEG activity, especially in the hippocampus, was considerably changed after administration of D-40TA in these transected preparations, the following criterion for determining the post-drug, hippocampal arousal threshold of the above stimulation was adopted: appearance of comparatively regular rhythms of slower frequency or faster waves of high amplitude. The latter pattern was obtained mostly in response by PH stimulation (Fig. 3). As shown in Figure 6, D-40TA in 0.5 mg/kg elevated selectively the hippocampal arousal thresholds at any stimulation without significantly affecting the neocortical arousal threshold.
FIG. 4. Effect of D-40TA on EEG arousal response to stimulation of the dorsomedial nuclei of the thalamus in curarized, cerveau isolé cat.
Abbreviations are as in Fig. 1.

Fig. 3. Effect of D-40TA on EEG arousal response to stimulation of the posterior hypothalamus in curarized, cerveau isolé cat.
Abbreviations are as in Fig. 1.

2. Sympathetic nerve (SN) and peripheral excitatory responses to sciatic nerve stimulation

In several cats, the contractile response of NM as well as the vasopressor response evoked by sciatic nerve stimulation were compared before and after brain-stem transec-
tion, and after subsequent injection of D-40TA in the same preparation. Electrical SN and PN efferent activities were also recorded in some preparations.

Before transaction, the contraction of NM associated with increased SN discharges and vasopressor response were readily obtained by sciatic stimulation at 1 or a lower voltage. The frequency and amplitude of PN volleys were also altered to some extent by stimulation, e.g., brief electrical silence followed by an increase in the frequency.

After the brain-stem transection, slight reduction in spontaneous SN activity, decreased frequency but prolonged duration of the spontaneous PN volleys and lower BP level were observed; furthermore, spontaneous sympathetic excitatory episodes, which occurred frequently in the intact brain preparations, were never observed. In this transected state, the vasopressor response was obtained with comparative ease by sciatic stimu-

**TABLE 4. Effect of D-40TA on the peripheral responses induced by stimulation of the sciatic nerve in curarized, cerveau isolé cats**

| Test agent | Stimulus intensity (volts) | NM       | BP        |
|------------|---------------------------|----------|-----------|
| D-40TA     |                           |          |           |
| 0.5 mg/kg  | pre-drug 0.18±0.04 (2)    | 0.52±0.13 (6) |
| i.v.       | post-drug 0.02±0* (2)     | 0.33±0.15 (6) |
| 4          | pre-drug 0.18±0.07 (4)    | 0.67±0.18 (5) |
|            | post-drug 0.02±0* (4)     | 0.38±0.18 (5) |

* Statistically significant from the pre-drug values at p<0.05 level.
Numerals in parentheses indicate number of cats used.

**FIG. 5. Effect of D-40TA on sympathetic excitatory responses to stimulation of the sciatic nerve in curarized, cerveau isolé cat.**

Integ. SN : integrated value of SN activity. Other abbreviations are as in Fig. 1.
lation but the degree was about half that observed before transection. Although the SN discharges were augmented by the stimulus, it was difficult to induce the contraction of NM even by the stimulus at a higher voltage. Actually, the height of contraction of NM induced by the stimulus at 2 or 4 volts after transection was about one-fifth that before transection.

Injection of D-40TA in 0.5 mg/kg i.v. to these cerveau isolés markedly reduced not only the augmented response of SN discharges but also the contractile response of NM to sciatic nerve stimulation, however, the vasopressor response was not significantly affected (Table 4, Fig. 5).

**Fig. 6.** Effect of D-40TA on the threshold of the neocortical and hippocampal EEG arousal responses induced by stimulation of the dorsomedial nuclei of the thalamus (TH) and posterior hypothalamus (PH) in curarized, cerveau isolé cats.

Vehicle (Control)  
D-40TA 0.5 mg/kg i.v.

Vertical lines represent the standard error. *: p<0.05.

**DISCUSSION**

D-40TA is a new central nervous system depressant which possesses potent sedative-hypnotic, tranquilizing, anticonvulsive properties qualitatively similar to chlordiazepoxide, diazepam and nitrazepam in various animal species. The results obtained in this study regarding the mechanism of sedative-hypnotic action of this compound will be discussed on the basis of the present neurophysiological knowledge relevant to the sleep mechanism.

1. **EEG arousal mechanism**

Neocortical EEG arousal threshold and duration: In the past two decades, extensive neurophysiological studies on the functions of the brain-stem reticular formation (4, 16) have proved an essential role of this structure for controlling the level of consciousness. The hypothalamus is also known to play a controlling role in consciousness and is considered to be a rostral end structure of the brain-stem activating system (17-20). Jasper's concept (5) of a diffuse thalamic projection system suggests a functional connection between this system and the midbrain reticular formation (MRF); thus diencephalic ends
of the reticular activating system are divided into the thalamic pathway (non-specific thalamic nuclei) and the extrathalamic pathway (hypothalamus and subthalamus). The neocortical EEG arousal has been proposed to consist of an initial, short-lasting "phasic" component directly related to awaking and a delayed, long-lasting "tonic" component for maintenance of wakefulness (8-10). Several studies by brain lesion, transection and stimulation (21-24) have demonstrated that both components are conducted from the midbrain to the neocortex mainly via the thalamic pathway but a "tonic" component is also enhanced by a powerful inflow of impulses from the hypothalamus to MRF. Furthermore, Bonvallet's group has postulated that a "phasic" component is due to the brief firing of the reticular neurons, whereas a "tonic" component would be the consequence of a delayed but self-sustained discharge of intrareticular neuronal circuits located in the dorsal part of the rostro-pontine tegmentum in the highest population (10, 25).

The present experiments revealed that D-40TA in 0.5 and 2 mg/kg showed little or no influence on the thresholds of the neocortical EEG arousal response, to either midbrain or sciatic stimulation in the intact brain cats and to thalamic stimulation in the cerveau isolés. These results clearly indicate that this compound does not have direct effect on the neocortical activating system via MRF and thalamic pathway. In contrast, the threshold of hypothalamic stimulation in the cats with intact brain as well as in the cerveau isolé cats was markedly elevated by D-40TA. Moreover, the duration of neocortical EEG arousals evoked by MRF, hypothalamic and sciatic stimulation at supra-threshold intensity in the intact brain cats was shortened by D-40TA and nitrazepam, regardless of the stimulation sites, to the degree that a "tonic" component was completely eliminated in some cases.

On the above neurophysiological basis, such selective suppression of a "tonic" component of the EEG arousal by D-40TA may be due not only to reduction of a tonic inflow via the hypothalamus into MRF but also to selective action on the intrareticular neurons relevant to sustained firings. The latter action is implicated by the fact that a "tonic" component generally accompanies sympathetic excitation (9) and the contraction of nictitating membrane by sciatic stimulation in the cerveau isolé cats was markedly suppressed by D-40TA.

**Hippocampal EEG arousal threshold:** It is established that the hippocampal EEG arousal characterized generally by synchronized theta waves is readily evoked by sensory stimulation via MRF and thalamic pathway in the cats with intact brain. Thalamic and hypothalamic stimulations also induce the hippocampal arousal response in association with neocortical desynchronization in the intact brain and even cerveau isolé cats. However, the rostral midbrain transected cats respond to hypothalamic stimulation at threshold intensity with ready appearance of the hippocampal arousal waves without a marked change in the neocortical EEG and conversely, to thalamic stimulation with marked desynchronization in the neocortex and less change in the hippocampus, activation of the latter requiring more intensive stimulation (22). These observations suggest that the hypothalamus constitutes an activating system to the hippocampus directly, and the back-
ground activity of the thalamus to send activating impulses to the hippocampus depends considerably on inflow from the midbrain itself as well as from the hypothalamic-midbrain pathway.

In our present study, slowing of spontaneous EEG with a decrease in normal theta waves by D-40TA in the cats with intact brain implicates a lowered activity of this structure. Furthermore, in the cerveau isolé cats leaving the hypothalamic-rostral midbrain connection intact, D-40TA markedly raised the hippocampal arousal thresholds of thalamic and particularly hypothalamic stimulation. Thus, it is more reasonable to consider that D-40TA suppresses the hippocampal activity directly and indirectly through a depressant action on the hypothalamus.

II. Central sympathetic excitatory mechanism

In the curarized cats with intact brain, sympathetic excitations characterized by augmented efferent discharges of the cervical sympathetic nerve, consequent contraction of nictitating membrane and the vasopressor response occurred frequently in a spontaneous way and by stimulation of MRF; hypothalamus and sciatic nerve. All these signs of the spontaneous sympathetic excitation were completely eliminated by electrolytic lesions of the bilateral posterior hypothalamus (26) or the brain-stem transection. Furthermore, the brain-stem transection diminished markedly, the contractile response of nictitating membrane to sciatic stimulation but only partly its vasopressor response. Thus, the augmented sympathetic efferent discharges and the contraction of nictitating membrane evoked spontaneously or by stimulation in the cats with intact brain appears to be a good index for evaluating the sympathetic excitatory outflow via the hypothalamus.

Like the hypothalamic lesion, the administration of D-40TA abolished almost completely development of the spontaneous sympathetic excitations in the cats with intact brain. Moreover, the thresholds of the augmented sympathetic discharges and peripheral responses to hypothalamic, midbrain and sciatic stimulations were all elevated by 2 mg/kg, like the same dose of nitrazepam, except that there was no effect on the vasopressor response to midbrain stimulation with D-40TA. The hypothalamic threshold was also elevated by the lower dose of D-40TA. Possibility that the peripheral responses are affected by D-40TA in the ganglion or peripheral receptor site can be excluded, since this compound at the dose level used here has been previously confirmed to have no effect on the contraction of nictitating membrane induced by preganglionic efferent stimulation of the cervical sympathetic nerve or on the vasopressor response to noradrenaline or adrenaline in the curarized spinal cats (3). Therefore, it is considered that depression of these sympathetic excitatory outflows by D-40TA occurs primarily in the hypothalamus itself or in the substrate located between this structure and the midbrain or both. Secondly, the depressant action of this compound on sympathetic substrates situated between the midbrain and the spinal sympathetic motoneuron in the lateral horn, perhaps on the midbrain neurons itself, also exists, since the contraction of nictitating membrane induced by sciatic stimulation in the cerveau isolé cats was markedly suppressed by this compound. However, the blood pressure regulating mechanism in the midbrain and medulla oblongata
appears to be resistant to D-40TA.

The present neuropharmacological data of D-40TA on sympathetic excitability strongly suggest that this compound may produce the sleep receptive condition for animals by reduction of emotional responsiveness. It was observed in a previous work (2) that most of the experimental animals, particularly monkeys, when given D-40TA fell into the sleeping state which was comparatively easily interrupted spontaneously or by external stimulation but was regained shortly after cessation of the stimulus. This fact is supported by the selective depressant action of this compound on the mechanism related to a "tonic" component of the EEG arousal, i.e., tonic inflow from the hypothalamus to MRF and also probably intrareticular self-sustained neurons, without significant influence on a "phasic" component related directly to awaking ability.

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