Abstract—Effects of intraventricularly administered serotonin (5HT), noradrenaline (NA), dopamine (DA) and metaraminol on the reserpine-induced spikes recorded from the medial nucleus Trapezoides (Trap. m.) in rabbits were investigated. 5HT (30, 50 µg) produced marked decreases in the amplitude and discharge rate of the spikes 3 to 5 min after intraventricular administration. NA (30, 50 µg) also produced similar effects to those of 5HT, but DA at the same dosage produced no significant changes in the amplitude and discharge rate of spikes. Metaraminol, a metabolite of α-methyl-meta-tyrosine, produced gradual and long-lasting, potent suppression of spikes. Ninety min later, spikes were completely suppressed, and no recovery was observed within 6 hours after intraventricular administration. These results indicate that NA has a similar suppressing action to that of 5HT on the generation of the reserpine-induced spikes.

Previously, we reported that distinct spikes could be recorded from the medial nucleus Trapezoides (Trap. m.) in rabbits during paradoxical sleep (PS) (1). Spikes similar in many aspects to spikes during PS were induced by reserpine administration continuously over 8 to 12 hr (2). Reserpine-induced spikes from the Trap.m. (reserpine-induced Tr spikes) were markedly suppressed by i.p. injection of 5-hydroxytryptophan (5HTP) (30, 50 mg/kg). The same dosage of 1,3,4-dihydroxyphenylalanine (L-DOPA) also produced slight but significant suppression on the reserpine-induced Tr spikes (3). These findings suggested that both 5HT and catecholamines exerted an inhibitory influence on the generation mechanisms of spikes.

In the present work, we attempted to determine whether the suppressing action of L-DOPA was due to the effect of DA or NA, by direct application of these amines to the lateral ventricle in rabbits. We also investigated the effect of metaraminol, a metabolite of α-methyl-meta-tyrosine (α-MMT), in order to elucidate the long lasting, potent suppressing action of α-MMT on the reserpine-induced Tr spikes described in the previous report (3).

MATERIALS AND METHODS

Male rabbits weighing 3.0 to 3.2 kg were used. Electrodes for recording the EEGs,
neck muscle electromyogram (EMG) and eye movement were chronically implanted by the same procedures as those described in previous reports (1–3). The stainless cranial cannula (outer diameter: 0.4 mm) for the drug administration was implanted stereotaxically into the anterior horn of the lateral ventricle. The correct placement of the cannula was verified by outflow of cerebrospinal fluid into the lumen of the cannula, when the core which had previously been inserted into the cannula was removed. The cannula was connected to a silicone rubber tube, and led out on the surface of the dental cement which fixed the electrodes and connector on the cranium. The tip of the silicone rubber tube was closed with a stainless steel stopper except during drug administrations. The drugs were dissolved or diluted in a small quantity of saline, the pH of the drug solution was adjusted to 6 to 7 by the addition of 0.1 N NaOH, and the drug concentration was then finally prepared so that 0.1 ml of solution would contain 30 to 50 μg of each drug just before the intraventricular administration.

Reserpine in a dose of 0.5 mg/kg produced spikes in the Trap.m. 2 to 3 hr after i.v. injection. When the amplitude and discharge rate of spikes were stable, the drugs were administered in a volume of 0.1 ml using a micro-syringe through a previously implanted cannula in freely moving animals. Residual drug solution in the cannula was expelled into the lateral ventricle by saline in a volume of 0.05 ml. Polygraphic recordings were made in a sound-attenuated room under conditions in which the animals could move freely. Behavior of the animals after drug administrations was monitored on a TV camera.

All experiments were performed at least 5 days after the implantation of the electrodes and injection cannula. After termination of all the experiments, methylene blue solution in an equal volume to that of the test drugs was administered through the same injection cannula in order to confirm the extent of spread of the drugs.

The drugs used were as follows: reserpine (Serpasil®, Takeda), serotonin creatinine sulfate (5HT, Sigma), dl-noradrenaline (NA, dl-norepírenamine®, Sankyo), dopamine hydrochloride (DA, Sigma) and metaraminol bitartrate (Araminon®, Banyu). Dosages of these drugs were expressed as free base. The statistical significance of the results was determined by Student’s t-test.

RESULTS

1. Effects of intraventricularly administered saline on the amplitude and discharge rate of reserpine-induced Tr spikes

Saline in a volume of 0.15 ml the pH of which was adjusted to 6 to 7 produced no change in the amplitude and as shown in Fig. 1, no significant change in the discharge rate of spikes was observed for 60 min as compared with the value before saline administration.

2. Effects of intraventricularly administered 5HT, NA, DA and metaraminol on the amplitude and discharge rate of reserpine-induced Tr spikes

1) Effects of 5HT: 5HT in a dose of 30 μg produced marked suppression in the amplitude and discharge rate of spikes 3 to 5 min after administration. Sixty minutes later, the amplitude and discharge rate of spikes recovered to about 50% of the "pre-drug"
FIG. 1. Effect of intraventricularly administered saline (0.15 ml) on the discharge rate of reserpine induced Tr spikes. Each point indicates the mean of 7 experiments. The ordinate shows the value represented as a percentage of the numbers of spikes during 5 min just before saline administration. Number of spikes (mean±S.E.) before saline administration was 372±47/5 min. The abscissa shows the time after saline administration.

FIG. 2. Effect of intraventricularly administered 5HT on the reserpine-induced Tr spikes. A typical example showing the effects of 30 μg of 5HT on the amplitude and discharge rate of spikes 3 and 60 min after 5HT administration.

Fig. 3. Time course of changes in the discharge rate of reserpine-induced Tr spikes after 5HT (30, 50 μg) administration. Each point indicates the mean of 5 and 4 experiments for 30 (filled circles) and 50 μg (open circles) of 5HT, respectively. The ordinate shows the value represented as a percentage of the numbers of spikes during 5 min just before drug treatments. Numbers of spikes (mean±S.E.) just before drug treatments were 332±55 and 300±63/5 min for 30 and 50 μg of 5HT, respectively. The abscissa shows the time after drug administration. Shaded area shows the range of standard deviation for saline control. Significantly different from saline control, **p<0.01 (t-test).

value (Fig. 2). The higher dose (50 μg) of 5HT showed a more potent suppressing action, but the time course of the recovery of the discharge rate of spikes was similar to that seen with the smaller dose (30 μg) (Fig. 3).

2) Effects of NA: NA in a dose of 30 μg produced marked suppression in the amplitude and discharge rate of spikes 3 to 5 min after administration. Sixty min later, the amplitude and discharge rate of spikes recovered to about 80% of the “pre-drug” value (Fig. 4). The higher dose of NA (50 μg) produced a more potent suppressing action. The time course
3) Effects of DA: DA in doses of 30 and 50 μg produced no change in the amplitude of spikes within a 60 min period. The discharge rate was slightly decreased for about 60 min, but this change was not significantly different as compared to that of saline control (p<0.05) (Fig. 6).

4) Effects of metaraminol: As shown in Figs. 7 and 8, metaraminol in a dose of 30 μg produced gradual decreases in the amplitude and discharge rate of spikes. Ninety minutes later, spikes were completely suppressed and no recovery was observed within 6 hr after metaraminol administration.

3. Effects of 5HT, NA, DA and metaraminol on the conscious state in rabbits pretreated with reserpine

Reserpine in a dose of 0.5 mg/kg produced marked behavioral changes including
FIG. 7. Effect of intraventricularly administered metaraminol on the reserpine-induced Tr spikes. A typical example showing the effects of 30 μg of metaraminol on the amplitude and discharge rate of spikes 5, 10, and 60 min after metaraminol administration.

FIG. 8. Time course of change in the discharge rate of reserpine-induced Tr spikes after metaraminol (30 μg) administration. Each point indicates the mean of 6 experiments. Number of spikes (mean ± S.E.) just before drug treatments was 320 ± 40/5 min. Significantly different from saline control, *p < 0.05, **p < 0.01 (t-test). For further explanations see Fig. 3.

sedation, ptosis, catalepsy etc. ("reserpine syndrome") over 12 hr, beginning 20 to 30 min after i.v. administration. In contrast to the deep sedation, a low voltage fast cortical EEG was observed for 2 to 3 hr after reserpine (an initial period of desynchronization). This phase of desynchronization was progressively followed by a depressive phase with slow wave and spindles in the cortical EEG (a period of synchronization). Spikes in the Trap.m. appeared 30 to 40 min after reserpine injection and continued for 8 to 12 hr without any association with the above described alternation of the period of desynchronization and synchronization. The onset of PS episode was delayed by more than 8 to 9 hr.

None of the drugs administered into the lateral ventricle produced polygraphic or behavioral changes, except for suppression of reserpine-induced Tr spikes, during the initial period of desynchronization or synchronization.

4. The extent of spread of drugs given intraventricularly

At the end of the experiments, methylene blue solution in an equal volume to the test drugs was administered through the cannula into the lateral ventricle and the extent of spread of the dye was assessed by gross examination.

When the brain was removed 5 to 10 min after injection of the dye solution, outer surface of the ventricular system (the lateral ventricle, the 3rd and 4th ventricles), the cisterna magna and the ventral surface of the brain stem were found to be colored by the dye.

DISCUSSION

We confirmed herein the potent suppressing action of 5HT on the reserpine-induced Tr spikes and that NA has a definite suppressing action of spikes. DA given in the same dosages as NA produced no significant changes in the amplitude and discharge rate of spikes. Therefore, the suppressing action of L-DOPA on the reserpine-induced Tr spikes
described in the previous report (3) may be due to the action of NA. In the same way, the facilitatory effect of \( \alpha \)-methyl-p-tyrosine (\( \alpha \)-MT) on the generation of reserpine-induced Tr spikes may be produced by decrease in NA in the brain (3). The results in this study also suggested that the delayed and long lasting suppressing action of \( \alpha \)-MMT reported previously (3) may be due to the action of the active metabolite, metaraminol, which probably increases NA concentration in the synaptic cleft by blocking the amine uptake mechanism of the presynaptic membrane of NA neurons (4, 5). The suppressing action of 5HT and NA rapidly appeared when such were administered into the lateral ventricle. Therefore, these actions may be the result of 5HT and NA, and not the deaminated metabolites of these amines, as suggested in the generation of the ponto-geniculo-occipital (PGO) spikes in cats (6, 7). In view of the extent of spread of the drugs in the brain and the rapid onset of action, the suppressing sites of action of NA were considered to be located in the vicinity of the wall of the ventricular system and subarachnoid space beneath the ventral surface of the brain stem. NA in doses employed in this experiment, produced no obvious changes in behavior ("reserpine syndrome") nor in the conscious state in the animals pretreated with reserpine. Therefore, the suppressing action of NA on the reserpine-induced Tr spikes might not be the result of change in the conscious state as Brooks and Gershon (8) postulated for the PGO spikes in cats.

Recently, we found that neurons in the ventral part of the Subnucleus pontis parvo-cellularis and Griseum pontis rostral to the Corpus Trapezoides were most sensitive to NA in suppressing the reserpine-induced Tr spikes (9).

Haefely et al. (10, 11) and Monachon et al. (12) suggested that the NA neuron system originating in the locus coeruleus exerts a tonic inhibitory influence on the generation of the PGO spikes in cats. Thus, more detailed sites of suppressing action of NA, similar to those seen in cases of 5HT, in the reserpine-induced Tr spikes remain to be elucidated.

It would thus appear that dual tonic inhibitory systems, one employing 5HT, and the other NA regulate the generation mechanisms of the spikes in the Trap.m. in a physiological condition, and release from the inhibitory systems by reserpine produces continuous emergence of the spikes. DA was considered to play no important role in the generation mechanisms of the spikes.

REFERENCES

1) Kimura, K.: Electroencephalographical studies on the hypnotic agents. Report 2. Effects of certain hypnotic agents on electrical activity in the lower brain stem of rabbits. *Folia pharmacol. japon.* **69**, 621-644 (1973) (*Abs. in English*)

2) Kimura, K.: Effects of monoamine-related compounds on the sleep-awake cycle in rabbits. *Folia pharmacol. japon.* **72**, 453-473 (1976) (*Abs. in English*)

3) Kimura, K., Kimura, Y., Ohata, K. and Takagi, H.: Effects of several monoamine-related compounds on the reserpine-induced spikes recorded from the medial nucleus Trapezoides in rabbits. *Japan. J. Pharmacol.* **28**, 317-327 (1978)

4) Iversen, L.L.: The uptake of catecholamines at the high perfusion concentrations in the rat isolated heart: A novel catecholamine uptake process. *Brit. J. Pharmacol.* **25**, 18-33 (1965)

5) Iversen, L.L.: Role of transmitter uptake mechanisms in synaptic neurotransmission.
6) Jones, B.E.: The respective involvement of noradrenaline and its deaminated metabolites in waking and paradoxical sleep. A neuropharmacological model. *Brain Res.* **39**, 121–136 (1972)

7) Jouvet, M.: The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergebn. Physiol.* **64**, 166–307 (1972)

8) Brooks, D.C. and Gershon, M.D.: An analysis of the effect of reserpine upon pontogeniculo-occipital wave activity in the cat. *Neuropharmacol.* **11**, 499–510 (1972)

9) Ukai, Y., Kimura, K., Ohata, K. and Takagi, H.: Sites of suppressing action of noradrenaline on the so-called "reserpine spikes". *Japan. J. Pharmacol.* **27**, Suppl. 41P, (1977)

10) Haefely, W., Jalife, M. and Monachon, M.A.: NE-neurons and phasic sleep phenomena. *Frontiers in Catecholamine Research*, Edited by Usdin, E. and Snyder, S.H., p. 773–775, Pergamon Press, Oxford (1973)

11) Haefely, W., Jalife, M. and Monachon, M.A.: A pathway and neurotransmitters involved in the control of noradrenergic locus coeruleus neurons. *Chemical Tools in Catecholamine Research*, Edited by Almgren, O., Carlsson, A. and Engel, J., Vol. 2, p. 135–142, North-Holland Pub. Comp. (1975)

12) Monachon, M.A., Jalife, M. and Haefely, W.: A modulating effect of chlordiazepoxide on drug-induced PGO spikes in the cat. *The Benzodiazepines*, Edited by Garattini, S., Masseini, E. and Randall, I.O., p. 513–529, Raven Press, New York (1973)