EFFECTS OF SEVERAL MONOAmine-RELATED COMPOUNDS
ON THE RESERpine-INDUCED SPIKES RECORdED FROM
THE MEdIAL NUCLEUS TRAPEZOIDES IN RABBITS

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Accepted November 30, 1977

Abstract—Effects of several monoamine-related compounds on the reserpine-induced
spikes recorded from the medial nucleus Trapezoides (reserpine-induced Tr spikes) in
rabbits were investigated. 5HTP (30-50 mg/kg, i.p.) showed marked suppression of
reserpine-induced Tr spikes. Parachlorophenylalanine (PCPA) alone induced spikes
similar to reserpine-induced Tr spikes after repeated doses (250 mg/kg twice daily for
3 successive days). A marked enhancement of the generation of reserpine-induced
Tr spikes was elicited, when reserpine was given to rabbits pretreated with PCPA.
L-DOPA (30-50 mg/kg, i.p.) showed a slight but significant suppressive action 20 to
40 min after injection. α-methyl-p-tyrosine (α-MT) (200 mg/kg, i.p.) produced no
significant change within 6 hr, but did show a marked facilitatory effect on the generation
of the spikes when reserpine was given to rabbits pretreated with α-MT. α-methyl-meta
tyrosine (α-MMT) (100 mg/kg, i.v.) caused a long-lasting, marked suppression. These
findings suggest that both catecholamines and 5HT have a suppressive action on the
generation of reserpine-induced Tr spikes.

Sleep consists of two grossly different stages from the point of view of neurophysiological
aspects—one is characterized by synchronous cortical EEG, called slow-wave sleep (SWS),
and the other is a paradoxical sleep (PS) or rapid eye movement (REM) sleep. PS has two
different phenomenological characteristics, namely, a tonic component including a desyn-
chronized cortical EEG, regular theta rhythm in the hippocampus and a total atonia of
antigravity muscles, and a phasic component including the ponto-geniculo-occipital (PGO)
spikes in cats (1-3). Above all, the PGO spikes are the most informative event for in-
vestigating the mechanisms of PS onset during SWS.

Matsumoto and Jouvet (4) found that reserpine in a single dose of 0.5 mg/kg, i.v. sup-
pressed SWS (for 12-14 hr) and PS (for 22-24 hr), while it induced continuous PGO spikes
independently of conscious states for 50 to 60 hr. Moreover they found that 5HTP (30-
50 mg/kg, i.p.) suppressed the PGO spikes, while the same dose of L-DOPA enhanced these
spikes and produced behavioral and polygraphic PS via a brief period of SWS. These
findings led to the investigation of the mechanisms of PS in the hope of demonstrating the
role of biogenic amines in priming and executive action of PS.

One of the authors (Kimura, K.), found that distinct spikes similar to the PGO spikes
in cats could be recorded from the medial nucleus Trapezoides (Trap. m.) in rabbits during
PS (5). In a physiological condition, these spikes (Tr spikes) usually preceded the other
signs of PS by several seconds. These findings suggested that Tr spikes might be associated with the triggering mechanisms of PS. Spikes similar in many aspects to Tr spikes were also induced by a single injection of reserpine (0.5 mg/kg, i.v.) continuously over 8 to 12 hr (6).

In the present work, we investigated the generation mechanisms of reserpine-induced Tr spikes with particular attention given to the relation to the role of biogenic amines.

**MATERIALS AND METHODS**

Male rabbits weighing 2.8 to 3.2 kg were used and all surgical procedures were performed under pentobarbital anesthesia. Bipolar stainless steel electrodes (0.2 mm in tip diameter) with a 0.5 mm vertical separation between tips were stereotaxically implanted in the Trap. m. bilaterally. The location of the tips of the electrodes was as follows: 13.5 to 13.8 mm posterior to the antero-posterior zero, 1 mm lateral to the sagittal suture, and 11.5 to 12.0 mm under the horizontal zero when the brains were fixed in the stereotaxic apparatus as described by Sawyer et al (7). A silver ball electrode (0.8 mm in diameter) was placed extra-durally over the motor cortex, and a stainless steel wire electrode was implanted in the dorsal hippocampus according to the stereotaxic coordinates of Sawyer et al (7). The EEG from the Trap. m. was recorded bipolarly and the time constant was set at 0.03 sec in order to pick up only the spike component. The EEGs from the motor cortex and the dorsal hippocampus were monopolarly recorded against the indifferent electrode placed over the frontal bone in the usually employed manner. Two stainless steel electrodes, the tips of which were bent like fishhooks were embedded into the neck muscle for recording the electromyogram (EMG). Fine silver wire electrodes with a 1 cm separation between the tips were placed near the canthus to record the eye movements.

Polygraphic recordings were made in a sound-attenuated room under conditions in which animals could freely move. All experiments were performed at least 5 days after the implantation of electrodes. After all the experiments were terminated, the locations of the tips of the electrodes in the Trap. m. were histologically checked with reference to the brain atlases of Meessen and Olszewski (8), and of Winkler and Potter (9).

Reserpine (Serpasil®, Takeda) and dl-α-methyl-m-tyrosine (α-MMT, NBC) were injected i.v. dl-5-hydroxytryptophane (5HTP, Sigma), 1-dihydroxyphenylalanine (L-DOPA, Sigma), dl-α-methyl-p-tyrosine (α-MT, Sigma) and parachlorophenylalanine (PCPA, Sigma) were suspended in 0.5% sodium carboxymethylcellulose solution and injected i.p. Behavior of the animals after drug administrations was observed on a TV camera. The statistical significance of the results was determined by Student’s t-test.

**RESULTS**

1. **Spikes in the Trap. m. during paradoxical sleep and after reserpine administration**

Polygraphic recordings at each conscious stage before and after reserpine administration are shown in Fig. 1A, B. At the beginning of PS, 2 or 3 isolated spikes, the amplitude of which was less than 50 μV appeared in the Trap. m. Gradually, 5 to 10 spikes appeared in bursts alternately from the right and left nuclei. During fully developed periods of PS,
more than 10 spikes appeared in bursts, and these spike bursts were usually followed by rapid eye movement (REM) bursts. Spikes similar to Tr spikes were induced 40 to 60 min after a single injection of reserpine (0.5 mg/kg). Such were at first isolated, but later grouped into bursts of 5 to 20 spikes. The amplitude of each spike was 25 to 100 μV and the duration was 30 to 80 msec. Two to three hours after reserpine (0.5 mg/kg), the amplitude and discharge rate of spikes were stable and such could not be distinguished from Tr spikes from many aspects. Reserpine-induced Tr spikes alternately appeared from the right and left nuclei, and the alternation was much more distinct compared to the Tr spikes. In some
cases, bizarre behavior with explosive jerky movements and REM bursts was observed periodically for 2 to 6 hr after reserpine administration. In these brief periods at the arrow in Fig. 1B, polygraphically similar aspects to those during REM bursts of PS were obtained.

![Graph showing effects of repeated reserpine treatment on the latent periods before the first appearance of reserpine-induced Tr spikes, and on the duration of occurrence of spikes.](image)

**TABLE 1. Effects of repeated reserpine treatments on the latent period before the first appearance of reserpine-induced Tr spikes (RS) and on the duration of occurrence of RS**

| Treatment | Drug  | Doses mg/kg (i.v.) | No. of experiments | Latent period of the first appearance of RS mean ± S.E. | Duration of occurrence of RS (hr) |
|-----------|-------|--------------------|--------------------|------------------------------------------------------|----------------------------------|
| 1st       | reserpine | 0.5                | 8                  | 45 min 38 sec ± 13 min 16 sec                        | <24                             |
| 2nd       | reserpine | 0.1                | 4                  | 7 min 40 sec ± 3 min 5 sec                           | <24                             |
| 3rd       | reserpine | 0.3                | 4                  | 5 min 36 sec ± 2 min 18 sec                          | <24                             |

Injections were given at 24 hr intervals.
Reserpine-induced Tr spikes were not seen 24 hr after a single injection of reserpine (0.5 mg/kg). When a smaller dose of reserpine (0.1–0.3 mg/kg) was subsequently injected 24 hr after the first injection, spikes reappeared after a brief period (5–10 min) and were sustained constantly for 6 to 8 hr. Similar results were obtained with further injection of the same dose of reserpine at an interval of 24 hr, though the duration of occurrence of Tr spikes produced by reserpine was gradually shortened. A typical example is shown in Fig. 2. The latent periods before the first appearance of spikes after repeated injection of reserpine are shown in Table 1.

2. Effects of several compounds which alter the brain catecholamine and 5HT levels on the reserpine-induced Tr spikes

The drugs were administered when the amplitude and discharge rate of spikes had stabilized after single or repeated doses of reserpine. In some cases, reserpine was injected into the animal pretreated with α-MT or PCPA.

1) 5HTP

Effects of 5HTP on the amplitude and discharge rate of the reserpine-induced Tr spikes are shown in Figs. 3 and 4. Marked decreases in the amplitude and discharge rate of spikes were observed 5 to 10 min after i.p. injection of 30 mg/kg of 5HTP. Spikes were completely inhibited 30 min after 5HTP injection in 2 out of 5 animals. The amplitude and discharge rate of spikes gradually recovered 40 min after 5HTP injection, but complete recovery was not obtained even 4 hr after 5HTP injection. In all animals treated with 50 mg/kg of 5HTP, almost complete suppression began 30 min after the injection and lasted for over 2 hr. In doses employed in this experiment, a slight predominance of slow wave sleep stage was observed for about 1 hr after the injection of 5HTP.

2) L-DOPA

L-DOPA in doses of 30 and 50 mg/kg did not produce any change in the amplitude of spikes, but did cause a slight but significant decrease in the discharge rate 20 to 40 min after injection of 30 and 50 mg/kg. However, dose related inhibition of the discharge rate of

![Fig. 3. Effects of 30 mg/kg (i.p.) of 5HTP on the amplitude and discharge rate of reserpine-induced Tr spikes.](image-url)
spike was not observed (Fig. 5). L-DOPA, in doses employed in this experiment, produced slight increases in the respiratory rate and predominance of arousal state for about 40 min after injection but produced no obvious changes in reserpine syndromes.

3) \( \alpha \)-MT

i) \textit{reserpine+} \( \alpha \)-MT: \( \alpha \)-MT injected to the animals 2 to 3 hr after a single dose of reserpine (0.5 mg/kg) produced no significant changes in the amplitude and discharge rate of spikes within 6 hr. In two animals which showed a slight decrease in the discharge rate 6 hr after \( \alpha \)-MT, additional injection of a small dose of reserpine (0.1 mg/kg) restored the discharge rate to the level before \( \alpha \)-MT treatment after a latent period of 5 to 7 min.
Figs. 6, 7).

FIG. 6. Effects of α-MT on the amplitude and discharge rate of reserpine-induced Tr spikes. Small dose of reserpine (0.1 mg/kg) was additionally given i.v. 6 hr after α-MT injection.

FIG. 7. Time course of changes in the discharge rate of reserpine-induced Tr spikes after α-MT (200 mg/kg, i.p.) and saline injection (i.p.). Each point indicates the mean of 5-8 experiments. The ordinate shows the value represented as a percentage of the numbers of spikes during 30 min just before drug treatments. Numbers of spikes (mean ± S.E.) before drug treatments were 2116 ± 148 (n=8) and 2606 ± 226 (n=5)/30 min for α-MT and saline, respectively. Abscissa: time in hr after α-MT and saline injection. No significant change from saline control was observed (t-test), at p<0.05. Numbers in parentheses represent number of experiments. ○ ○○: α-MT 200 mg/kg, i.p. ● ●●: saline, i.p.

Figs. 6, 7).

ii) α-MT+reserpine: Since the discharge rate of reserpine-induced Tr spikes was considerably irregular and gradually decreased 6 to 8 hr after reserpine injection, animals pretreated with α-MT were used in order to investigate the delayed and long lasting action of α-MT. A single injection of reserpine in a dose of 0.1 mg/kg (i.v.) usually produced no spikes, but did produce distinct spikes when it was injected into animals pretreated with α-MT (200 mg/kg, i.p.) 8 hr prior to reserpine injection. When a higher dose of reserpine (0.5 mg/kg) was injected, the latent period of the first appearance of spikes was significantly shortened, compared to the control pretreated with saline (Table 2). Furthermore, bizarre behavior with explosive jerky movements and REM bursts was observed at 30 min concomitantly with the occurrence of spikes of high amplitude and marked discharge rate. These polygraphic and behavioral changes periodically appeared for 6 to 8 hr after reserpine
injection. The period over which reserpine-induced Tr spikes appeared was markedly prolonged and in these animals, reserpine syndromes such as sedation, ptosis, myosis and catalepsy were observed even 28 hr after a single injection of reserpine 0.5 mg/kg.

4) \(\alpha\)-MMT

\(\alpha\)-MMT in a dose of 100 mg/kg (i.v.) caused marked decreases in the amplitude and discharge rate of reserpine-induced Tr spikes in 1.5 to 2 hr. Reserpine-induced Tr spikes were almost completely suppressed within 4 hr and no recovery was observed even 12 hr

| Pretreatment | Reserpine mg/kg (i.v.) | No. of experiments | Latent period before the first appearance of RS | Duration of occurrence of RS (hr) |
|--------------|------------------------|--------------------|---------------------------------------------|-----------------------------|
| Saline       | 0.1                    | 8                  | —                                            | —                          |
|              | 0.5                    | 8                  | 45 min 38 sec ± 13 min 16 sec\(^{(b)}\)       | <24                        |
| \(\alpha\)-MT\(^{**}\) (200 mg/kg) | 0.1                    | 4                  | 22 min 36 sec ± 6 min 8 sec                    | <24                        |
|              | 0.5                    | 4                  | 9 min 1 sec ± 1 min 34 sec\(^{(a)}\)          | >28                        |
| PCPA\(^{***}\) (250 mg/kg twice daily for 3 successive days) | 0.5                    | 4                  | 14 min 55 sec ± 2 min 3 sec\(^{(c)}\)          | >28                        |

\(^{*}\)A single dose of reserpine 0.1 mg/kg (i.v.) did not induce RS. **Reserpine was administered 8 hr after \(\alpha\)-MT. ***Reserpine was administered 72 hr after the last dose of PCPA. (a) Significantly different from the saline pretreated group (b) (t-test), P<0.05.

**Fig. 8.** Effect of \(\alpha\)-MMT on the amplitude and discharge rate of reserpine-induced Tr spikes. A small dose of reserpine (0.1 mg/kg) was additionally given i.v. 6 hr after \(\alpha\)-MMT injection.
after α-MMT injection in all animals observed. In contrast to the cases with α-MT, additional injection of reserpine (0.1 mg/kg) failed to induce the reappearance of spikes (Figs. 8, 9).

**FIG. 9.** Time course of changes in the discharge rate of reserpine-induced Tr spikes after α-MMT (100 mg/kg, i.v.) and saline (i.v.). Each point indicates the mean of 4–5 experiments. The ordinate shows the value represented as a percentage of the numbers of spikes during 30 min just before drug treatments. Numbers of spikes (mean ± S.E.) before drug treatments were 2122 ± 124 (n=5) and 2066 ± 170 (n=4); 30 min for α-MMT and saline, respectively. *Significantly different from saline control (t-test), p<0.05. For further explanations see Fig. 6.

5) **PCPA**

PCPA in a dose of 250 mg/kg was injected i.p. twice daily for 3 consecutive days. PCPA alone induced spikes similar to Tr spikes and/or reserpine-induced Tr spikes 16 hr after the last dose. These spikes were not observed during the arousal stage. When reserpine in a dose of 0.5 mg/kg was injected i.v. 72 hr after the last dose of PCPA, the latent period before the first appearance of reserpine-induced Tr spikes was significantly shortened compared to the control pretreated with saline. The spikes were detected even 28 hr after reserpine injection. Results are summarized in Table 2.

**DISCUSSION**

Small doses of 5HTP produced marked suppression of reserpine-induced Tr spikes. PCPA which is a specific depletor of 5HT (10) induced distinct spikes similar to Tr spikes seen during PS or after reserpine injection, and this compound also produced a marked facilitating effect on the generation of reserpine-induced Tr spikes. These findings in rabbits suggest that 5HT has a definite suppressing action on the generation of reserpine-induced Tr spikes.

In this experiment, L-DOPA also showed a slight but significant suppressive action on the discharge rate of reserpine-induced Tr spikes. α-MT, a specific depletor of catecholamines (11, 12) showed a facilitating effect on the generation of reserpine-induced Tr spikes. These findings with L-DOPA and α-MT suggest that catecholamines also have an inhibitory effect on the generation of reserpine-induced Tr spikes. Long-lasting, complete suppressive action of α-MMT on the reserpine-induced Tr spikes may not be due to the further depletion
of catecholamines as a result of inhibition of the enzyme aromatic L-amino acid decarboxylase (13, 14). α-MT, a specific depletor of catecholamines, showed rather facilitating effects on the generation of reserpine-induced Tr spikes, therefore, the suppressive action of α-MMT may be due to accumulation of the active metabolite, α-methyl metatyramine or metaraminol (13–17). We already confirmed that metaraminol has a long lasting potent suppressive action on the reserpine-induced Tr spikes when injected into the lateral ventricle (20). Metaraminol is considered to potentiate the action of noradrenaline (NA) by blocking the amine uptake mechanism of the presynaptic membrane of NA neurons (18, 19).

These findings suggest that NA may have a suppressive action on the generation of reserpine-induced Tr spikes and the reserpine-induced Tr spikes may be controlled under the dual tonic inhibitory action of 5HT and catecholamine-neuron (probably NA neuron) systems. Matsumoto and Jouvet (4) first reported that L-DOPA increased the frequency of the PGO spikes in reserpinized cats and often induced PS via brief periods of slow wave sleep. Brooks and Gershon (21) recently reported that similar results to those of Matsumoto and Jouvet (4) were not forthcoming in their own carefully controlled experiment. Haefely et al (22) and Monachon et al (23) also presented results providing strong evidence for the inhibitory action of the NA neuron system on the PGO spikes in cats.

The monoaminergic mechanisms responsible for the generation of the reserpine-induced Tr spikes in rabbits and the PGO spikes in cats may therefore be similar. We propose a hypothesis similar to that of Haefely et al (22). The activity of “pacemaker cells” which is responsible for the generation of the spikes is probably regulated by dual tonic inhibitory influences of 5HT and catecholamines, in such a way that the depletion of both 5HT and catecholamines by reserpine causes a release from the inhibition in the “pacemaker cells”. Consequently, the spontaneous firing of the “pacemaker cells” occurs. The excitatory impulses originating from the “pacemaker cells” may then produce synchronous discharges in the pool of the cells in the Trap. m. The structures responsible for the existence and location of the “pacemaker cells”, and those which exert a tonic inhibitory influence on the “pacemaker cells” remain to be elucidated in further studies.

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