EFFECTS OF CATECHOLAMINERGIC OR 
TRYPTAMINERGIC AGENTS ON THE MORPHINE-INDUCED 
STRAUB TAIL REACTION

Tsutomu KAMEYAMA, Makoto UKAI and Toshitaka NABESHIMA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, 
Meijo University, Tempaku-ku, Nagoya 468, Japan

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Abstract—An investigation was made of monoamine-related agents on the Straub tail reaction (STR) due to central excitatory effect of morphine (10 mg/kg). Apomorphine (10 mg/kg) showed a tendency to enhance the STR, and L-dopa (150 mg/kg) and methamphetamine (5 and 10 mg/kg) produced a significant increase in the STR. Phenoxybenzamine and propranolol induced the inhibition of the STR to a large extent. Diethylthiocarbamate (250 mg/kg) and disulfiram (200 and 400 mg/kg) had no influence on the STR. α-Methyl-p-tyrosine (100 and 400 mg/kg) reduced the STR. L-5-Hydroxytryptophan [L-5-HTP] (100 mg/kg) inhibited the STR significantly. Isocarboxazid (50 mg kg), nialamide (50 and 100 mg/kg) and tranylcypromine (10 and 25 mg/kg) did not influence the STR. On the contrary, biochemical investigations showed that morphine (10 and 20 mg/kg) decreased the 5-hydroxytryptamine (5-HT) content of the lumbar sacral cord significantly, although no alteration of 5-HT content occurred in various sites of the brain, compared to a vehicle-treated group. Morphine (10 and 20 mg/kg) did not act on the dopamine or norepinephrine content of various sites of the brain and spinal cord. Our results suggest that the STR is, at least to some extent, the result of an increase in catecholaminergic activity and/or a decrease in tryptaminergic activity in the central nervous system of mice.

The Straub tail reaction [STR] (1) is due to stimulation of the spinal cord (2). Gaddum (3) has suggested that this phenomenon is due to a spasm of the vesical and anal sphincters resulting from spinal stimulation. Bilbey et al. (4) suggested that the STR is due to contraction of the sacroccocygeus dorsalis muscle. We have proposed that the central nervous system (CNS) may play a significant role in the manifestation of the STR due to morphine in mice (5-7). Recently, studies directed towards investigating the exact mechanism by which morphine exerts its analgesic effect have focussed on the influence of the biogenic amines. Considerable work in this field has been done on the catecholaminergic or tryptaminergic system (8). However, there is little information available as to the relationship between the STR induced by morphine and biogenic amines in mice.

The present study was an attempt to clarify the effects of biogenic amines such as catecholamines and 5-hydroxytryptamine (5-HT) within the central nervous system (CNS) on the STR. In addition, motor coordination (MC) was measured together with the STR in order to exclude the influence of motor paralysis.

MATERIALS AND METHODS

Albino male mice of ddY strain weighing between 18 and 25 g were separated into
groups of ten to twenty. Food and water were provided ad libitum. The drugs used were as follows: morphine hydrochloride (Shionogi and Co., Ltd.), apomorphine (Sandoz), p-chlorophenylalanine (pCPA) (Nakarai Chemicals, Ltd.), disulfiram (Wako Pure Chemical Industries, Ltd.), L-dihydroxyphenylalanine (L-dopa) and L-5-hydroxytryptophan (L-5-HTP) (Kyowa Hakko Kogyo Co., Ltd.), diethyldithiocarbamate (DDC) and phenoxybenzamine (Tokyo Kasei Kogyo Co., Ltd.), 6-hydroxydopamine (6-OHDA), α-methyl-p-tyrosine (α-MT), propranolol and tranylcypromine (Sigma), haloperidol and methamphetamine (Dainippon Pharmaceutical Co., Ltd.), isocarboxazid (Takeda Chemical Industries Co., Ltd.), nialamide (Pfizer Taito Co., Ltd.) and tetrabenazine (Hoffmann-LaRoche). These drugs were dissolved in 0.9% saline or suspended in isotonic 0.3% carboxymethylcellulose solution immediately before use. 6-OHDA was injected directly into the brain of conscious mice according to reported methods (9, 10). The STR was observed 30 min after the administration of morphine 10 mg/kg, s.c. Tail elevation was graded according to the modified numerical ratings of Juul as follows: 0=0°, 0.5=1-44°, 1.0=45°, 1.5=46-89°, 2.0=90°, 2.5=91-179°, 3.0=180° above the horizontal table plane (11). In addition, the percent of increase or decrease in the STR was calculated as follows: A (the mean score of animals treated with morphine alone), B (the mean score of animals treated with monoamine related agents plus morphine). Percent of increase or decrease = \(\frac{A-B}{A} \times 100\). MC was measured by a rotarod apparatus (12). The test was conducted on trained mice (maximum of two trials). If the animal stayed on the rotarod less than 120 sec, the test was considered positive for motor incoordination. Furthermore, the inhibitory percent of MC was calculated as follows: \([\frac{\text{the number of animals positive}}{\text{the number of animals tested}}] \times 100\). For biochemical analysis, the mouse brain was dissected into four regions consisting of the cortex, cerebellum, diencephalon and mesencephalon, and pons plus medulla oblongata as described by Glowinski and Iversen (13) and the spinal cord was dissected according to the method of Sidman et al. (14). Brains and spinal cords were homogenized in 3 ml of ice cold 0.4 N perchloric acid and after centrifugation, dopamine, norepinephrine and 5-HT contents were assayed fluorometrically according to the method of Karasawa et al. (15). The statistical significance of the results was determined by Student's t-test for the STR and biochemical studies and by the Fisher exact probability test for MC. The experiments were programmed in a semi-soundproof room at 23 ± 1°C and 55 ± 2.5% relative humidity.

RESULTS

Effects of catecholamine-related agents and their combinations

As shown in Fig. 1, L-dopa 100 and 150 mg/kg, i.p. enhanced the STR but L-dopa 200 mg/kg, i.p. inhibited it strongly and was accompanied by marked motor incoordination. Apomorphine (10 mg/kg, s.c.), a dopamine receptor agonist, showed a tendency to increase the STR, and methamphetamine (5 and 10 mg/kg, i.p.), a releaser of catecholamines, produced a significant increase in the STR. Stereotyped behavior induced by methamphe-
tamine (10 mg/kg, i.p.) was more intense than that induced by methamphetamine (5 mg/kg, i.p.). In addition, apomorphine showed 50% inhibition of MC, but methamphetamine did not inhibit MC. When α-MT, a tyrosine hydroxylase inhibitor, and 6-OHDA, a drug which is used extensively to produce a selective degeneration of catecholaminergic neurons in experiments were employed, α-MT (100 mg/kg) showed a decrease in the STR, in contrast to α-MT plus L-dopa (100 mg/kg), which showed a tendency to increase the STR. 6-OHDA (100 μg/mouse, i.e.) had no influence on the STR. L-Dopa (100 mg/kg, i.p.) in combination with 6-OHDA (100 μg/mouse, i.c.) produced a significant increase in the STR. Tetrabenazine (40 mg/kg, i.p.) inhibited the STR completely and produced marked inhibition of MC. L-Dopa (100 mg/kg, i.p.) decreased the tetrabenazine-induced inhibition of the STR. None of the catecholamine related agents alone influenced the STR or MC, except methamphetamine which showed mild STR.

**Effects of catecholamine receptor blockers and catecholamine synthesis enzyme inhibitors**

In the next experiment, the effects of phenoxybenzamine and propranolol, adrenergic blocking agents, on the STR were investigated. As shown in Fig. 2, phenoxybenzamine (10 mg/kg, i.p.) inhibited the STR significantly. On the other hand, propranolol 25 mg/kg, i.p. had no influence on the STR while 50 mg/kg, i.p. decreased the STR significantly. However, propranolol 50 mg/kg, i.p. produced marked inhibition of MC. DDC 250 mg/kg, s.c. and disulfiram (200 and 400 mg/kg, p.o.), dopamine-β-hydroxylase inhibitors, had
no influence on the STR but DDC 500 mg/kg, s.c. produced a decrease in the STR and strong inhibition of MC. DDC (500 mg/kg, s.c.) alone showed fairly strong inhibition of MC. α-MT (100 and 400 mg/kg, i.p.) showed 55 and 65% inhibition of the STR without causing motor incoordination. Haloperidol (5 and 10 mg/kg) which seems to have a dopamine receptor blocking action, inhibited the STR significantly in the doses shown. Haloperidol (10 mg/kg, i.p.) inhibited MC to a considerable degree.

Effects of 5-hydroxytryptamine (5-HT)-related agents and their combinations

As indicated in Fig. 3, L-5-HTP (50, 100 and 200 mg/kg, i.p.), a precursor of 5-HT, exhibited 10, 24 and 34% inhibition of the STR, and L-5-HTP (200 mg/kg, i.p.) showed marked inhibition of MC. 6-OHDA (100 µg/mouse, i.c.) produced an increase in the L-5-HTP-induced inhibition of the STR [no statistical significance vs. L-5-HTP (100 mg/kg)-treated animals] accompanied by strong inhibition of MC. pCPA (2 × 300 mg/kg, i.p.) slightly decreased the L-5-HTP-induced inhibition of the STR [not significant vs. L-5-HTP (100 mg/kg)-treated animals]. On the other hand, pCPA-induced motor incoordination was facilitated by this combination (P<0.05 vs. pCPA alone). Tetrabenazine (35 mg/kg) had only a slight influence on the STR and increased the L-5-HTP-induced inhibition of the STR [P<0.05 vs. L-5-HTP (100 mg/kg)-treated animals].

Effects of monoamine oxidase inhibitors

As indicated in Fig. 4, isocarboxazid (50 mg/kg, i.p.), nialamide (50 and 100 mg/kg, i.p.), and tranylcypromine (10 and 25 mg/kg, i.p.) produced no significant changes in the STR.
Effects of morphine on brain and spinal cord dopamine content in mice

As shown in Table 1, morphine (10 and 20 mg/kg, s.c.) did not alter the dopamine content in various brain regions or the spinal cord when compared to vehicle-treated control values.

Effects of morphine on brain and spinal cord norepinephrine content in mice

As shown in Table 2, there was no change in various brain regions or the spinal cord treated with morphine (10 and 20 mg/kg, s.c.) when compared to vehicle-treated control.
Effects of morphine on brain and spinal cord 5-hydroxytryptamine (5-HT) content in mice

As shown in Table 3, there was no change in the regions of the cerebral cortex, cerebellum, mesencephalon, or pons + medulla oblongata except for the lumbosacral cord in which morphine produced a significant reduction in 5-HT content in a dose-dependent fashion.

| Table 1. Effects of morphine on brain and spinal cord dopamine content |
|-----------------------------|---------------|----------------|----------------|----------------|----------------|
| Region                      | Cortex        | Cerebellum     | Mesencephalon  | Pons + Medulla | Lumbosacral cord |
| Dose (mg/kg)                |               |                |               | oblongata      |                |
| 0                           | 0.59 ± 0.06   | 0.09 ± 0.05    | 0.52 ± 0.06   | 0.13 ± 0.01    | 0.08 ± 0.02    |
| 10                          | 0.64 ± 0.02   | 0.05 ± 0.03    | 0.48 ± 0.05   | 0.14 ± 0.01    | 0.07 ± 0.02    |
| 20                          | 0.68 ± 0.03   | 0.09 ± 0.03    | 0.43 ± 0.02   | 0.16 ± 0.01    | 0.08 ± 0.03    |

Either saline or morphine was injected s.c. 30 min before decapitation. Values of dopamine content represent mean ± S.E. μg/g wet tissue.

| Table 2. Effects of morphine on brain and spinal cord norepinephrine content |
|-----------------------------|---------------|----------------|----------------|----------------|----------------|
| Region                      | Cortex        | Cerebellum     | Mesencephalon  | Pons + Medulla | Lumbosacral cord |
| Dose (mg/kg)                |               |                |               | oblongata      |                |
| 0                           | 0.20 ± 0.01   | 0.14 ± 0.01    | 0.44 ± 0.01   | 0.38 ± 0.01    | 0.25 ± 0.01    |
| 10                          | 0.22 ± 0.01   | 0.14 ± 0.03    | 0.44 ± 0.02   | 0.35 ± 0.01    | 0.23 ± 0.03    |
| 20                          | 0.19 ± 0.01   | 0.13 ± 0.02    | 0.41 ± 0.01   | 0.39 ± 0.01    | 0.30 ± 0.04    |

Either saline or morphine was injected s.c. 30 min before decapitation. Values of norepinephrine content represent mean ± S.E. μg/g wet tissue.

| Table 3. Effects of morphine on brain and spinal cord 5-hydroxytryptamine (5-HT) content |
|-----------------------------|---------------|----------------|----------------|----------------|----------------|
| Region                      | Cortex        | Cerebellum     | Mesencephalon  | Pons + Medulla | Lumbosacral cord |
| Dose (mg/kg)                |               |                |               | oblongata      |                |
| 0                           | 0.52 ± 0.04   | 0.06 ± 0.02    | 0.84 ± 0.05   | 0.42 ± 0.02    | 0.63 ± 0.05    |
| 10                          | 0.52 ± 0.01   | 0.05 ± 0.02    | 0.82 ± 0.05   | 0.48 ± 0.03    | 0.45 ± 0.04*   |
| 20                          | 0.53 ± 0.03   | 0.06 ± 0.02    | 0.85 ± 0.07   | 0.54 ± 0.03    | 0.39 ± 0.03*** |

Either saline or morphine was injected s.c. 30 min before decapitation. Values of 5-HT content represent mean ± S.E. μg/g wet tissue. *P<0.05; ***P<0.01 when compared to values in the group given saline.

Effects of morphine on brain and spinal cord 5-hydroxytryptamine (5-HT) content in mice

As shown in Table 3, there was no change in the regions of the cerebral cortex, cerebellum, mesencephalon, or pons + medulla oblongata except for the lumbosacral cord in which morphine produced a significant reduction in 5-HT content in a dose-dependent fashion.

DISCUSSION

Previously, it has been speculated that the STR is evoked through the mediation of the CNS (5-7). The present work was an attempt to determine the effects of several biogenic amines within the CNS on the STR. L-Dopa (150 mg/kg, i.p.) provided a significant increase in the STR. Apomorphine showed a tendency to enhance the STR. Methamphetamine (5 mg/kg, i.p.) produced a significant increase in the morphine-induced STR in comparison with that of methamphetamine (10 mg/kg, i.p.). These findings suggest that
the greater the activity of stereotypy, the less the STR increasing effect of methamphetamine. It has been reported that apomorphine appears to stimulate central dopamine receptors directly (16, 17) and that amphetamine as well as methamphetamine facilitates the release of catecholamines (18, 19). L-Dopa strongly antagonized the inhibitory action of the STR induced by α-MT or tetrabenazine alone. It is also known that the supersensitivity (20) due to 6-OHDA is evoked at the postsynapse of catecholaminergic neurons in the CNS. 6-OHDA (100 μg/mouse, i.e.) had no influence on the STR but L-dopa plus 6-OHDA (100 μg/mouse, i.e.) showed a significant increase in the STR, probably due to postsynaptic supersensitivity induced by 6-OHDA. With respect to the central stimulant effects of amphetamine, Barchas et al. (21) have stated that phenoxybenzamine, an α-adrenergic blocking agent, is partially able to antagonize the action of amphetamine. Phenoxybenzamine and propranolol (β-blocker) prevented the STR significantly. Propranolol also significantly inhibited MC. On the other hand, a dopamine-β-hydroxylase inhibitor reportedly increases dopamine content but greatly decreases norepinephrine content (22). In addition, Spector et al. (23) suggested that α-MT decreases catecholamine content in the brain, i.e., the brain stem and caudate nucleus. DDC and disulfiram had no influence on the STR while α-MT did show an intense inhibition of the STR. Moreover, haloperidol which seems to block dopamine receptors, produced a significant decrease in the STR. In the biochemical study, the various sites of the brain and spinal cord sampled were unresponsive to morphine with respect to dopamine and norepinephrine contents.

It may be hypothesized that catecholaminergic agents facilitated the morphine-induced STR by stimulating catecholaminergic pathways (by release of catecholamines and/or inhibition of re-uptake processes and/or direct receptor stimulation) antagonized by catecholaminergic blocking agents. The differences in efficacy of catecholaminergic agents may partly be a reflection of their differential rates of metabolism by monoamine oxidase (24).

The effects of increase or decrease in the activity of 5-HT within the CNS on the STR were also determined. L-5-HTP markedly inhibited the STR in a dose-dependent fashion. Moreover, pCPA, a compound which inhibits tryptophan hydroxylase (25), showed a slight decrease in L-5-HTP-induced inhibition of the STR. This result may be partially compatible with the effects of L-5-HTP in combination with pCPA on the monosynaptic reflex described by Taber and Anderson (26). L-5-HTP plus 6-OHDA showed a tendency to increase the inhibitory percent of the STR compared to L-5-HTP alone, but this was not significant. The STR as well as MC appeared to be prevented by tryptaminergic agents but pCPA (2 × 300 mg/kg, i.p.) showed a slight increase in the STR despite the marked inhibition of MC. This result suggests that the involvement of tryptaminergic agents on the STR is more selective than that on MC.

In addition to the results of catecholaminergic drugs on the STR, the attenuation of the STR by the injection of L-5-HTP suggests that the STR is, at least in part, caused by an increase in catecholaminergic activity or a decrease in tryptaminergic activity in the CNS of mice.

It is well known that monoamine oxidase inhibitors increase both catecholamines and
5-HT (27), but when the animals were given isocarboxazid, nialamide and tranylcypromine, the STR was not influenced. These findings suggest that the STR may be modulated by a variation in the activity of either catecholamines or tryptamine within the CNS. Tetrabenazine, a depletor of monoamines (28-30), inhibited the STR in contrast to the effects of monoamine oxidase inhibitors. However, the fact that the tetrabenazine-induced inhibition of the STR was attenuated by the effect of L-dopa, but facilitated by L-5-HTP, indicates the occurrence of fluctuations in the monoamine activity balance in the brain.

Biochemical investigations show that morphine decreases the 5-HT content of the lumbosacral cord dose-dependently, although there are no alterations in the 5-HT content of various sites of the brain, compared to a vehicle-treated group. Thus, the lumbosacral cord may be one site of action where the balance between levels of catecholamines and tryptamine may play an important role in the manifestation of the STR.

On the other hand, L-dopa, apomorphine, 6-OHDA, propranolol, haloperidol, DDC, L-5-HTP and tetrabenazine had a considerable degree of inhibition on both MC and the STR in moderate to high doses, while other drugs scarcely inhibited MC. This finding supports the hypothesis that the larger the dose administered the greater the extent of motor incoordination. Consistent information as to the relationship between the STR and MC was not obtained as the rotarod test, unlike the STR, involves psychic components such as attention, learning and memory, which unavoidably accompany an experimental test based on training (see Materials and Methods section) (31). We are currently investigating the relationship between the STR and MC from this point of view.

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