EFFECTS OF CHLORPROMAZINE, IMIPRAMINE AND BACLOFEN ON THE SPINAL POLYSYNAPTIC REFLEX IN ACUTE, CHRONIC AND 6-HYDROXYDOPAMINE-TREATED SPINAL RATS

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Accepted July 12, 1982

Abstract—Effects of the drugs affecting monoaminergic neurotransmission were examined on the spinal polysynaptic reflex (PSR) in anesthetized spinal rats. Chlorpromazine HCl (0.5–2.0 mg/kg, i.v.) and baclofen (0.63–2.5 mg/kg) depressed and imipramine HCl (1.25–5.0 mg/kg) increased the amplitude of PSR in acute spinal animals, recorded as evoked electromyogram in the gastrocnemius muscle by electrical stimulation of the common peroneal nerve. However, chlorpromazine and imipramine showed effects neither on PSR in chronic spinal rats, nor in acute spinal rats with the intracisternal administration of 6-hydroxydopamine, which caused depletion of the spinal noradrenaline, dopamine and serotonin, and selective depletion of the spinal noradrenaline, respectively. Baclofen depressed the amplitude of PSR in both preparations with almost the same potency as that in acute spinal ones. Imipramine HCl (2.5 mg/kg, i.v.), chlorpromazine HCl (1.0 mg/kg) and baclofen (1.25 mg/kg) depressed the mono- and polysynaptic heights of the ventral root potentials in acute spinal rats. However, their depression of polysynaptic height was not so strong. These observations strongly suggest that, at the receptor sites on spinal interneurons where the descending monoaminergic neurons terminate, spinal monoamines, especially noradrenaline, are involved in PSR modification by chlorpromazine and imipramine, but not in PSR depression by baclofen.

Descendino monoamine (MA) neurons terminating in the ventral and dorsal regions of the spinal cord (1–3) regulate its neuronal function via the MA receptors existing on the interneurons and motoneurons (4–6). Chlorpromazine which has been suggested to block the postsynaptic dopamine and noradrenaline (NA) receptors in the brain and spinal cord (4, 5) depresses spinal reflexes (4, 7, 8). Imipramine inhibits the presynaptic reuptake of NA and serotonin (9, 10). Clineschmidt et al. (6) reported that imipramine potentiates the 5-hydroxytryptophan-induced facilitation of the monosynaptic reflex via central interaction with serotonin formed from 5-hydroxytryptophan. Fukuda et al. (11) suggested that baclofen depresses spinal reflex through an involvement of catecholamine. Thus, these drugs seemed to give a clue for elucidating the relationships between MA neurons and spinal cord functions.
In the present experiments, to elucidate the involvement of MA in spinal interneuron activity, the effects of these three drugs affecting MA neurotransmission were examined on spinal polysynaptic reflex (PSR) in rats with acutely transected spinal cord; and as the result, chlorpromazine and imipramine were found to depress and facilitate the PSR, respectively. Therefore, the effects on PSR in chronic spinal and 6-hydroxydopamine (6-OHDA)-treated spinal rats were compared with those in acute spinal ones since chronic spinal transection pronouncedly decreases MA content in the spinal cord below the transection (12–14) and 6-OHDA intracisternally administered leads to depletion of the endogenous NA in the spinal cord (15, 16).

A preliminary report of these findings was published previously (17).

MATERIALS AND METHODS

General procedure: Male Wistar HLA rats weighing 250 g to 400 g were used. They were housed in temperature and humidity-controlled rooms with a 12/12 hr light-dark cycle, 5 in a cage, and fed food and water ad libitum. Animals were anesthetized with combined intraperitoneal injection of α-chloralose (50 mg/kg) and urethane (400 mg/kg). Thereafter, supplementary doses of the anesthetics were given during the course of the experiments. Under the anesthesia, several peripheral nerves were exposed at the popliteal fossa, and the common peroneal nerve was dissected. The animal, fixed in a stereotaxic apparatus (Todai Noken type), was clamped at the spine and tibial bone. After additional anesthesia with diethylether, the rat was spinalized at the cervical region (CI) using a spatula, and artificially ventilated with room air at a rate of 60 strokes/min. The exposed neural tissues were covered with warm liquid paraffin, and body temperature was maintained constant at 36.5±0.5°C by an infrared lamp. A cannula was inserted into the femoral vein for the administration of drugs.

Recording of spinal reflex: A concentric needle electrode was inserted into the ipsilateral gastrocnemius muscle, and a stimulating electrode (interelectrode distance 4.5 mm) placed on the central end of the common peroneal nerve at the popliteal fossa. Square wave pulses of 0.1 msec duration supra-maximal in strength via an isolation unit were applied once every 10 sec for eliciting the evoked electromyogram (EMG). The evoked EMG activities, amplified with an AC amplifier (Nihon Kohden RB2), were displayed on a cathode ray oscilloscope (Nihon Kohden VC9). The amplitude of the evoked EMG was measured on photographs of five superimposed oscilloscope tracings. In some experiments, ventral root potentials (VRP) were recorded in acute spinal rats. After the laminectomy, a recording electrode (inter-electrode distance 4.5 mm) was placed on the L5 ventral root, and stimuli applied to the common peroneal nerve or L5 dorsal root via an isolation unit. Stimulus conditions were the same as those in the evoked EMG experiments. In addition, arterial blood pressure in acute spinal rats was recorded from the right carotid artery by means of a transducer (Nihon Kohden MP-4T) and displayed on an inkwriting oscillograph.

Preparations of 6-OHDA-treated rats: Animals deeply anesthetized with diethylether were mounted on a stereotaxic head holder with the head bent. Through a needle (diameter 0.5 mm) introduced into the cisterna magna, 10 μl of saline containing 50 μg 6-OHDA (as a base) and 2 μg ascorbic acid was infused over 3 min. After the operation, an antibiotic drug was injected to prevent infection. Thereafter, the rats were housed in individual cages. These animals were used for the biochemical and pharmacological examinations 10–20 days later.
Preparations of chronic spinal rats: Under diethylether anesthesia, a dorsal laminectomy was performed at the thoracic level. The spinal cord (T8) was completely transected with self-made spatula under visual control. The wound was closed and antibiotic drug applied locally. Thereafter, the rats were housed in individual cages, and their bladder was emptied twice a day and cleaned with sterile alcohol solution. These animals were used for the examinations 10–20 days later.

Measurements of brain and spinal cord MA contents: Brain and spinal cord MA contents in 6-OHDA-treated rats and chronic spinal rats were determined in comparison with those in sham operated control rats. The animals were sacrificed by decapitation, and the brain and spinal cord quickly removed. Determination of NA, dopamine and serotonin in the tissues was performed fluorometrically using the method previously described (18).

Drugs: All drugs were injected intravenously into a cannulated femoral vein over a 30-sec period after the spinal reflex had remained stable over 15–30 min. Chlorpromazine HCl and imipramine HCl were used in a commercial injectable form. Baclofen was dissolved in 0.9% saline before the injection.

Statistics: For statistical evaluation of the reflex responses, the amplitude obtained in each rat was expressed as a percentage of the pre-injection value, and the mean values were calculated from 5–8 separate experiments. Statistical analyses of the drug effects were carried out by the Student’s t-test.

RESULTS

Characteristics of evoked EMG: Electrical stimulation of the common peroneal nerve caused an evoked EMG response in the gastrocnemius muscle in anesthetized spinal rats (Fig. 1). The latency from the stimulation to the beginning of the evoked EMG was 7.96±0.08 msec (mean±S.E.M., n=15) in acute spinal animals. This latency was almost the same as that in spinal animals with 6-OHDA treatment (7.94±0.16 msec, n=15), but longer than that in chronic spinal ones (6.84±0.07 msec, n=15). The amplitude of evoked EMG in 6-OHDA-treated spinal and chronic spinal animals was larger than that in acute spinal ones. In addition, the threshold voltage for eliciting the evoked EMG in 6-OHDA-treated spinal and chronic spinal rats was lower than that in acute spinal ones.

When recorded from the L5 ventral root by

Fig. 1. Effects of chlorpromazine, imipramine and baclofen on evoked EMG in acute spinal rats. The evoked EMG was recorded from the gastrocnemius muscle by electrical stimulation of the common peroneal nerve.
the stimulation of the common peroneal nerve, the latencies of mono- and polysynaptic components of VRP were 2.64±0.01 msec and 4.30±0.10 msec, respectively (mean±S.E.M., n=5). In addition, the latency of the M wave, recorded from the gastrocnemius muscle by stimulation of the tibial nerve, was 1.34±0.05 msec (n=7).

MA contents in 6-OHDA-treated rats and chronic spinal rats: NA, dopamine and serotonin contents in the spinal cord and brain tissues of 6-OHDA-treated animals or chronic spinal animals were compared with those in sham operated control ones (Table 1). Spinal NA content in 6-OHDA-treated animals was 25.9±12.8 ng/g wet weight.

Table 1. Monoamine contents in spinal cord and brain tissues of intact and 6-OHDA treated animals

| No of animals | Intact rats | 6-OHDA treated rats |
|---------------|-------------|---------------------|
|               | 5           | 10                  |
| NA            | 249.6±24.6 ng/g (100.0±9.9) | 25.9±12.8** ng/g (10.4±4.9) |
| Spinal Cord   |             |                     |
| DA            | 64.3±10.0 (100.0±16.6) | 52.1±8.5* (81.0±13.2) |
| 5-HT          | 360.6±84.5 (100.0±22.8) | 344.1±54.5 (92.9±14.7) |
| NA            | 320.5±12.2 (100.0±3.8) | 205.8±31.4** (64.2±9.8) |
| Brain         |             |                     |
| DA            | 823.4±29.6 (100.0±3.6) | 817.3±72.0 (99.3±8.8) |
| 5-HT          | 379.2±25.6 (100.0±6.8) | 393.7±31.0 (103.8±8.2) |

Abbreviations: NA=noradrenaline, DA=dopamine, 5-HT=serotonin. Differences statistically significant from the value of intact animals: *P<0.05 and **P<0.01 (Student's t-test).

Fig. 2. Effects of chlorpromazine and imipramine on evoked EMG in acute spinal rats. Ordinate: evoked EMG amplitude, expressed as a percentage of preinjection value. Abscissa: time after injection (min). Each point represents the mean in 5–8 separate experiments with the S.E.M. indicated. Differences from the control that are statistically significant: *P<0.05 and **P<0.01 (Student’s t-test).
and its value was 10.4% of the control animals (P<0.01, t-test). In 6-OHDA-treated animals, spinal serotonin content was almost the same as that in control ones, while dopamine content slightly decreased (P<0.05). In the brain tissues, NA content decreased to 64.2% of that in control animals (P<0.01), while dopamine and serotonin contents were the same in both preparations. In the spinal cord below the transection of chronic spinal rats, NA, dopamine and serotonin contents were less than each minimum detectable concentration.

Effect on evoked EMG: Chlorpromazine HCl (1.0 mg/kg, i.v.) decreased the amplitude of evoked EMG in acute spinal rats (Fig. 1). On the other hand, imipramine HCl (2.5 mg/kg) increased the amplitude gradually. Baclofen (2.5 mg/kg) decreased the amplitude with longer duration. As shown in Fig. 2, chlorpromazine HCl (0.5–2.0 mg/kg) decreased the amplitude of evoked EMG, and imipramine HCl (2.5, 5.0 mg/kg) increased it up to about 150%, 10 min later. Baclofen in a dose range of 0.63–2.5 mg/kg dose-relatedly depressed the evoked EMG (Fig. 3). The effects of these three drugs were statis-

Fig. 3. Effect of baclofen on evoked EMG in acute spinal rats. Ordinate: evoked EMG amplitude, expressed as a percentage of preinjection value. Abscissa: time after injection (min). Each point represents the mean in five separate experiments with the S.E.M. indicated. Differences from the control that are statistically significant: *P<0.05 and **P<0.01 (Student’s t-test).

Fig. 4. Effects of chlorpromazine, imipramine and baclofen on evoked EMG in chronic spinal and 6-OHDA-treated spinal rats. Ordinate: evoked EMG amplitude, expressed as a percentage of preinjection value. Abscissa: time after injection (min). Each point represents the mean in five separate experiments with the S.E.M. indicated. Differences from the control that are statistically significant: *P<0.05 and **P<0.01 (Student’s t-test).
tically significant from the saline control.

In 6-OHDA-treated spinal rats, chlorpromazine HCl (1.0 mg/kg) and imipramine HCl (2.5 mg/kg) were without effect on the amplitude of the evoked EMG. However, baclofen (1.25 mg/kg) depressed the evoked EMG to the same extent as in the acute spinal rats (Fig. 4A). In the chronic spinal rats, the effects of chlorpromazine HCl (1.0 mg/kg), imipramine HCl (2.5 mg/kg) and baclofen (1.25 mg/kg) on evoked EMG were almost the same as those in 6-OHDA-treated spinal rats (Fig. 4B).

Effect on VRP in acute spinal rats: Chlorpromazine HCl (1.0 mg/kg, i.v.) depressed the mono- and polysynaptic heights of VRP

Fig. 5. Effects of chlorpromazine, imipramine and baclofen on ventral root potential in acute spinal rats.

Fig. 6. Effects of chlorpromazine, imipramine and baclofen on ventral root potential in acute spinal rats. Ordinate: monosynaptic and polysynaptic heights of ventral root potential, expressed as a percentage of preinjection value. Abscissa: time after injection (min). Each point represents the mean in five separate experiments with the S.E.M. indicated. Differences from the control that are statistically significant: *P<0.05 and **P<0.01 (Student's t-test).
Imipramine HCl (2.5 mg/kg) slightly depressed the mono- and polysynaptic heights, while baclofen (1.25 mg/kg) depressed the mono-synaptic height more strongly than the polysynaptic height.

**Effect on arterial blood pressure in acute spinal rats:** Chlorpromazine HCl (1.0 mg/kg, i.v.) slightly raised the mean blood pressure (6 mmHg) after the injection and returned it to the control level within 5 min. Imipramine HCl (2.5 mg/kg) decreased the blood pressure (12 mmHg) after the injection and raised it up to 20 mmHg, 3 min later. The effect was returned to the control level within 15 min. Baclofen (2.5 mg/kg) also decreased the blood pressure (19 mmHg) and returned it to the control level within 3 min. The changes in blood pressure and the effects on evoked EMG induced by the drugs seemed independent since the effects on the blood pressure were not always parallel to those on the evoked EMG.

**DISCUSSION**

PSR, recorded as the evoked EMG in the gastrocnemius muscle by electrical stimulation of the common peroneal nerve, was found in acute spinal rats to be depressed with the administration of chlorpromazine and baclofen and markedly facilitated with imipramine. There is no report with the clear facilitation of PSR by imipramine alone. Under the same conditions, these three drugs depressed the mono- and polysynaptic heights of the VRP in acute spinal rats, supporting the findings on VRP previously reported with chlorpromazine (8), imipramine (6), and baclofen (11). These effects were different from those on the evoked EMG in that imipramine facilitated the evoked EMG, and the effects of chlorpromazine and baclofen on the polysynaptic height of VRP were not so strong as those on the evoked EMG. The polysynaptic components of the VRP in rats seem to be a disynaptic reflex, based on the latency between the mono- and polysynaptic components. The difference between the latency of the evoked EMG and total latencies of the M wave and the polysynaptic component of the VRP was 2.32 msec. Therefore, the evoked EMG is considered to be one of extensor reflexes via two or more interneurons. Thus, the observations that imipramine facilitated the evoked EMG and slightly depressed the mono- and polysynaptic heights of the VRP strongly suggest that the interneurons positioned in the pathway eliciting the evoked EMG play an important role in PSR facilitation by imipramine. Similarly, it may be possible that chlorpromazine and baclofen also modify the activity of such interneurons.

The depression of the evoked EMG by chlorpromazine was neither observed in 6-OHDA-treated spinal rats where spinal NA was selectively depleted, nor in chronic spinal rats where spinal NA, dopamine and serotonin were completely depleted. Andén et al. (4) reported that chlorpromazine blocks NA and dopamine receptors in the spinal cord and depresses L - DOPA - induced facilitation of the flexor reflex. Our result strongly suggests that MA, especially NA, is involved in PSR depression by chlorpromazine. In the present experiments, spinal dopamine content in 6-OHDA-treated animals decreased, although slightly, indicating that the blockage of dopamine receptor by chlorpromazine may contribute to PSR depression. However, apomorphine, a dopamine stimulant, depressed the evoked EMG in acute spinal and chronic spinal rats (unpublished observation). Therefore, it seems unlikely that dopamine is involved in PSR depression by chlorpromazine.

Imipramine was without effect on the evoked EMG in chronic spinal rats, while the drug markedly facilitated it in acute spinal ones. Clineschmidt (19) reported that the
imipramine-induced facilitation of the mono-
synaptic reflex in acute spinal cats with MA
oxidase inhibitor was not observed in chronic
spinal ones with the inhibitor. Our result is in
line with his observation. Sinclair and Sastry
(20) reported that imipramine antagonized the
recurrent inhibition of the extensor mono-
synaptic reflex by enhancing the action of
either serotonin or NA through presynaptic
uptake inhibition. In the present experiments,
the facilitation of the PSR by imipramine was
blocked with the intracisternal treatment of
6-OHDA which selectively depleted spinal
NA content without changing serotonin
content. Therefore, our observation suggests
that the presynaptic reuptake inhibition of
spinal NA by imipramine is involved in PSR
facilitation and that serotonin has little
involvement in this phenomenon.

Fukuda et al. (11) reported from the
experiments with MA-depleted rats that
catecholamine seems involved in the de-
pressive effect of baclofen on the spinal
reflex. In the present experiments, however,
baclofen depressed the evoked EMG in
acute, chronic and 6-OHDA-treated spinal
rats; and the effectiveness was almost the
same in the three preparations. Therefore,
little involvement of MA is suggested in PSR
depression by baclofen. In addition, a direct
action of baclofen on the MA receptors may
disputed since supersensitivity of the
receptors has been demonstrated to develop
following the chronic spinal transection and
6-OHDA treatment of rats (15, 21).

We have been concerned with the con-
tribution of the descending MA neurons to
the spinal PSR, an extensor reflex, and
comparisons of the effects of the drugs
affecting MA neurotransmission in acute,
chronic and 6-OHDA-treated spinal rats. As
the results, spinal MA, especially NA, was
found, at the receptor sites on spinal inter-
neurons where the descending MA neurons
terminate, to contribute presumably to chlor-
promazine-induced depression and imipra-
mine-induced facilitation of PSR, but not to
baclofen-induced depression. In addition, it
is suggested that NA facilitates some kind
of polysynaptic pathway. The observation
supports the results of Dhawan and Sharma
(22) who reported that intrathecally adminis-
tered NA facilitates the flexor reflex. Further-
more, the interneurons involved in NA
neurotransmission seem different from those
existing in the polysynaptic pathway of the
VRP in the responsiveness to the drugs
and the location.

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