Some Correlations between Procaine-Induced Convulsions and Monoamines in the Spinal Cord of Rats

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Abstract—The relationships between the convulsions induced by the local anesthetic procaine and monoamines in the spinal cord were investigated in rats. The levels of dopamine (DA) and serotonin (5-HT) in the spinal cord were time dependently increased after procaine (170 mg/kg, i.p.), which induced clonic convulsions, but the level of norepinephrine (NE) was unchanged. The rats that died during convulsions had a marked increase in DA. Phenobarbital (25 mg/kg, s.c.) produced both depletion of DA and elevation of 5-HT in the spinal cord and completely protected rats against convulsions. A 5-HT precursor, 5-hydroxytryptophan (5-HTP; 20–80 mg/kg, i.p.), suppressed the convulsions in a dose dependent manner as shown by a decrease in the incidence and a prolongation of the latency, but a DA precursor, 3,4-dihydroxyphenylalanine (L-DOPA; 20–80 mg/kg, i.p.), markedly shortened the latency. Furthermore, the effect of L-DOPA on the convulsions was inhibited by the combination of 5-HTP. α-Methyl-p-tyrosine (20–80 mg/kg, i.p.) or DL-p-chlorophenylalanine (20–80 mg/kg, i.p.), an inhibitor of NE and DA or 5-HT biosynthesis, had a slight effect on the convulsions. These results suggest that procaine causes significant elevations of rat spinal DA and 5-HT in the convulsive process and suggest that dopaminergic and serotonergic neurons may be associated with procaine-induced convulsions.

It is generally known that local anesthetics have toxic reactions which may cause stimulation of the central nervous system (CNS), producing restlessness and tremor that may proceed to clonic convulsions (1).

Frank and Sanders (2) have suggested that the apparent stimulation and the subsequent depression produced by applying local anesthetics are due solely to depression of neuronal activity, and their convulsant effects are produced by a selective depression of inhibitory neurons, thereby allowing enhancement of the effect of excitatory neurons.

Thus, several investigators began pursuing a possible correlation between local anesthetic-induced convulsions and monoamines as inhibitory neurotransmitters in the CNS (3–11). However, the results obtained so far have been equivocal. For example, Ciarlone and Juras (8) reported that lidocaine induced depletion of DA and 5-HT in some regions of rat brain and elevation in others; however, it produced elevated levels of NE. Procaine induced elevations of NE, DA and 5-HT; NE elevation occurred in a few brain parts of rats, whereas DA and 5-HT levels were elevated in all brain regions. On the other hand, the results obtained by Gage and Dorris (6) indicated that lidocaine had no significant effect on DA levels in mouse brain. However, they did report increased DA in whole brain when mice were injected with tetracaine. These differences may point out that there are some differences in the chemical transmitters involved in the mechanisms of local anesthetic-induced convulsions or that the various local anesthetics may have different pharmacological effects in the CNS.

The monoaminergic system in the spinal cord would also be important in the mechanisms of local anesthetic-induced convulsions, but a functional role of monoamines in
the spinal cord in the procaine-induced convulsive process has not yet been clearly defined.

The present study was undertaken to establish whether or not there is a correlation between procaine injection and alteration of monoamines in the spinal cord and to investigate the influence of monoamine manipulation in the CNS on the incidence and the latency of procaine-induced convulsions in rats.

Materials and Methods

Animals: Male rats of the Wistar strain, weighing 170–190 g, were used. They were maintained on standard laboratory chow (Oriental Yeast Co., MF) and tap water ad libitum under the condition of constant room temperature (23–25°C) with a 12 hr light-dark cycle for at least 5 days before being used. The rats were fasted overnight before the experiment, but water was given until the time of the experiments.

Materials: L-3,4-dihydroxyphenylalanine methyl ester HCl (L-DOPA), DL-α-methyl-p-tyrosine methyl ester HCl (α-MPT), DL-p-chlorophenylalanine methyl ester HCl (PCPA), 5-hydroxytryptophan (5-HTP), 3,4-dihydroxybenzylamine HBr (DHBA), 5-hydroxytryptamine creatinine sulfate complex, dopamine HCl and procaine HCl were the products of Sigma Chemical Co. Sodium phenobarbital and norepinephrine HCl were obtained from Sankyo Pharmaceutical Co. and Nacalai Tesque Ltd., respectively. All other chemicals used were of analytical reagent grade.

Treatment of drugs: L-DOPA, α-MPT, PCPA and procaine were dissolved in distilled water and 5-HTP was suspended in olive oil. These drugs were administered at their appropriate dosages in a volume of 0.1 ml/100 g body weight. The test times of drugs are presented in the tables and figures.

Observation of convulsions: After procaine was administered to rats, the convulsive behavioral changes were recorded as follows: running motions of the limbs; tonic arching of the tail; back and neck; respiration; characteristics of convulsions (clonic/tonic); incidence of convulsions; onset time (latency) of convulsions; duration of convulsions; recovery or death. The experimental observation periods were for at least 1 hr.

Extraction and analysis of monoamines from rat spinal cord: Control or drug-treated rats were killed at designated times by decapitation; and the spinal cords were quickly removed, weighed, frozen in acetone and dry ice, and stored at −40°C until analyzed. Also, the spinal cords of rats that had died during convulsions were removed at the moment of death and frozen rapidly. All analyses were done within 2 days of each experiment.

The spinal cord was homogenized in 1 ml of 10 mM phosphate buffer (pH 6.3) containing DHBA (200 ng) as an internal standard. 2 ml of acidified n-butanol and 4 ml of n-heptane with a polytron homogenizer. After the suspension was centrifuged at 15,000×g for 30 min at 4°C, the aqueous layer of the supernatant was adjusted to pH 6.0–6.5 with 1.0 N NaOH and applied to a column of cellulose phosphate ion exchanger (approx. 45 mg, Whatman), which had previously been treated with 0.5 M phosphate buffer (pH 2.0). Then, the column was washed with 0.1 ml of distilled water and 0.2 ml of 0.5 M phosphate buffer (pH 2.0), and the adsorbed monoamines were subsequently eluted with 0.5 ml of 0.5 M phosphate buffer (pH 2.0) containing 1.0 M NaCl.

To determine NE, DA and 5-HT, 10–20 µl of the eluate was injected into a high performance liquid chromatograph (Yanaco L-2000) equipped with an electrochemical detector (ECD; Yanaco VMD-101).

The analysis was performed by separation on a reverse phase column (Nucleosil 7C18, 4.6×250 mm), with a mobile phase of 0.1 M phosphate buffer (pH 3.0) containing 8% methyl alcohol, pumped at a flow rate of 0.8 ml/min. The potential of the ECD was set at 0.8 V vs. an Ag/AgCl reference electrode. Recoveries of monoamines after pretreatment with the mixed adsorbent were: NE, 81%; DA, 83%; 5-HT, 93%, and DHBA, 94%.

Statistics: Significant differences between experimental groups of data were analyzed by Student’s t-test.

Results

Dose-response relationship between procaine dosage and convulsions: As shown in
Table 1. Relationships between the dose of procaine and the convulsive activity in rats

| Dose (mg/kg, i.p.) | No. of rats | No. of convulsions | Latency of onset (min) | Duration of convulsions (min) | Mortality |
|-------------------|-------------|--------------------|-----------------------|-----------------------------|-----------|
| 130               | 8           | 3                  | 7.55±2.18             | 7.29±1.24                   | 0         |
| 150               | 8           | 6                  | 6.87±1.32             | 9.63±1.66                   | 0         |
| 170               | 8           | 8                  | 5.48±0.68             | 14.23±1.64                  | 0         |
| 190               | 8           | 8                  | 4.50±0.73             | 16.01±2.60                  | 6         |

Each value represents the mean±S.D. *Time to death after convulsions.

Fig. 1. Effects of procaine on the levels of monoamines in the spinal cord of rats. Rats were given procaine (170 mg/kg, i.p.) and then killed 2, 4, 8.5, and 30 min later. **Rats that died during the convulsive activity after administration of procaine (190 mg/kg, i.p.). Each column represents the mean±S.D. of 8 to 10 rats. *P<0.05, **P<0.01, compared with the control values. *P<0.05, **P<0.01, compared with the values of procaine-treated groups.
Rats injected with 190 mg/kg of procaine assumed the opisthotonic position with head and neck bent backward and extension of the limbs. After this, the rats gasped for air, and many rats died during their convulsions. Thus, in the subsequent experiments, procaine was used at a dose of 170 mg/kg, i.p.

Monoamine levels in rat spinal cord after administration of procaine: From the convulsive behavioral changes after administration of procaine in rats, the following classifications of their behavioral state were made: 1) nonconvulsive state (2 min after administration), 2) preconvulsive state (4 min), 3) convulsive state (8.5 min) and 4) depressive state (30 min).

As shown in Fig. 1, the levels of NE in the spinal cord were not changed after administration of procaine, except the levels were increased only slightly during the preconvulsive state. The levels of DA were time-dependently increased from the nonconvulsive state after procaine. Although a significant increase was still observed during the depressive state, the maximum increase was observed during the preconvulsive state at 4 min after administration. The levels of 5-HT were also significantly increased from the nonconvulsive state to the depressive state after procaine, but the maximum increase was different from that of the DA levels. The maximum DA level was observed during the convulsive state at 8.5 min after administration. Interestingly, the rats that died during convulsions had a marked increase in DA to about 2.8 times the control level. However, the rats showed no marked changes in NE and 5-HT levels.

Monoamine levels in rat spinal cord after administration of phenobarbital: Phenobarbital pretreatment at a dose of 25 mg/kg, s.c. completely protected rats against convulsions induced by procaine at doses of 170 and 190 mg/kg, i.p. (data not shown). As shown in Fig. 2, phenobarbital also produced a signifi-

![Fig. 2](image)
cant decrease in DA and an increase in 5-HT in the spinal cord, 60 min after injection. The decrease in DA and the increase in 5-HT were 30% and 26% of the control values, respectively. Furthermore, these levels of DA and 5-HT were not changed after the additional administration of procaine. Phenobarbital had no significant effects on NE levels in the spinal cord, and NE levels were also unchanged after administration of procaine.

Effects of monoamine-related drugs on procaine-induced convulsions and monoamine levels in rat spinal cord: The results are summarized in Tables 2 and 3. As compared with the control rats, the latency of procaine-induced convulsions was dose-dependently shortened by pretreatment with L-DOPA, and the mortality was markedly increased. While i.p. administrations of monoamine-related drugs do not selectively act in the spinal cord, L-DOPA treatment produced a significant increase in DA to 147-246% of the control levels in the spinal cord, but caused only a slight increase in NE levels.

On the contrary, pretreatment with 5-HTP resulted in no convulsions in 20-30% of the procaine-treated rats and significantly prolonged the latency of convulsions with increasing concentration of 5-HT in the spinal cord, without altering NE and DA contents. Furthermore, the latency of convulsions was more prolonged after the combined administration of L-DOPA and 5-HTP than after L-DOPA alone. However, the combination of L-DOPA and 5-HTP produced no change in the incidence of convulsions and the mortality.

Pretreatment with α-MPT, an inhibitor of tyrosine hydroxylase, produced depletions of the concentrations of NE and DA in the spinal cord without changing the 5-HT levels, but had a slight effect on the incidence and the latency of procaine-induced convulsions. Although a similar decrease in 5-HT concentrations in the spinal cord was produced by pretreatment with PCPA, an inhibitor of tryptophan hydroxylase, the incidence and the latency of convulsions in PCPA-treated rats were without effect. However, 20-40% of α-MPT-treated rats and 40-60% of PCPA-

| Drug  | Dose (mg/kg) | No. of rats | Convulsions | Latency of onset (min) | Mortality |
|-------|-------------|-------------|-------------|------------------------|-----------|
|       |             |             | No. of rats | %                      | No. of rats | %       |
| Control | -           | 9           | 9           | 100                    | 4.88±1.21 | 0       |
| L-DOPA  | 20          | 6           | 6           | 100                    | 3.92±0.57* | 4       | 67  |
|        | 40          | 6           | 6           | 100                    | 3.05±0.35** | 4     | 67  |
|        | 80          | 6           | 6           | 100                    | 2.65±0.36** | 6     | 100 |
| α-MPT   | 20          | 6           | 6           | 100                    | 5.31±1.72 | 0       |
|        | 40          | 5           | 5           | 100                    | 4.60±0.86 | 1       | 20  |
|        | 80          | 5           | 5           | 100                    | 4.52±0.51 | 2       | 40  |
| 5-HTP   | 20          | 5           | 4           | 80                     | 5.63±1.54 | 0       |
|        | 40          | 5           | 4           | 80                     | 10.73±1.29** | 0 | 0 |
|        | 80          | 6           | 4           | 67                     | 10.85±2.07** | 0 | 0 |
| PCPA    | 20          | 5           | 5           | 100                    | 4.87±1.26 | 2       | 40  |
|        | 40          | 5           | 5           | 100                    | 4.75±0.91 | 3       | 60  |
|        | 80          | 5           | 5           | 100                    | 5.07±1.39 | 2       | 40  |
| L-DOPA  | 40          |             |             |                        |           |         |
| + 5-HTP |             | 20          | 5           | 5                      | 3.69±0.93 | 3       | 60  |
|         |             | 40          | 5           | 5                      | 4.92±0.81*** | 3 | 60  |
|         |             | 80          | 5           | 5                      | 6.57±1.07*** | 3 | 60  |

Rats were intraperitoneally injected with L-DOPA, 5-HTP, α-MPT and PCPA at 1, 1, 4 and 72 hr before being challenged with procaine (170 mg/kg, i.p.), respectively. Each value represents the mean±S.D. *P<0.05, **P<0.01, compared with the control values. ***P<0.01, compared with the values of L-DOPA (40 mg/kg)-treated groups.
Table 3. Effects of monoamine-related drugs on the levels of monoamines in the spinal cord of rats

| Drug  | Dose (mg/kg) | NE (ng/g tissue) | DA (ng/g tissue) | 5-HT (ng/g tissue) |
|-------|--------------|-----------------|-----------------|-------------------|
|       |              | 24 h            | 48 h            | 72 h              |
| Control | —             | 484±26          | 239±24          | 461±40            |
|       | 20            | 517±29          | 352±55**        | 436±39            |
|       | 40            | 533±42*         | 389±49**        | 467±80            |
|       | 80            | 544±20**        | 588±172**       | 447±38            |
| α-MPT | 20            | 375±47**        | 176±18**        | 487±18            |
|       | 40            | 339±23**        | 170±19**        | 462±38            |
|       | 80            | 351±35**        | 186±33*         | 469±64            |
| 5-HTP | 20            | 479±89          | 228±18          | 704±65**          |
|       | 40            | 488±26          | 233±28          | 1039±88**         |
| PCPA  | 20            | 510±27          | 247±25          | 412±18*           |
|       | 40            | 492±21          | 230±11          | 327±28**          |
|       | 80            | 495±27          | 253±27          | 146±10**          |

Rats were intraperitoneally treated with either L-DOPA, 5-HTP, α-MPT or PCPA and then sacrificed 1, 1, 4 and 72 hr later. Each value represents the mean±S.D. of 5 to 7 rats. *P<0.05, **P<0.01, compared with the control values.

treated rats died during convulsions after administration of procaine.

Discussion

This paper has shown some interesting correlations between procaine injection and monoamine levels in rat spinal cord and the possibility of an involvement of spinal DA and 5-HT, as well as brain monoamines, in the procaine-induced convulsive process.

The correlation between procaine-induced convulsions in rats and alterations of monoamine levels in the brain has been studied in detail by Ciarlone and Juras (8) and Kawano (10). They reported that convulsive doses of procaine induced elevations of monoamine steady-state levels in the brain, and it produced widespread elevations of DA and 5-HT in all brain regions with progressing convulsive activity. In the present study, we have also observed that the levels of DA and 5-HT in the spinal cord were time-dependently increased after administration of procaine at the convulsive dose (Fig. 1). Gage and Dorris (6) also reported that the local anesthetic tetracaine produced convulsions in mice and significantly elevated DA levels in the brain. However, they found that if tetracaine-induced convulsions were blocked by pretreatment of mice with diazepam, DA levels were still elevated. Their results indicate that the convulsions by tetracaine per se were not causally related to the rise in brain DA levels after injection. However, when we pretreated rats with phenobarbital, we prevented procaine-induced convulsions, and this treatment prevented significant elevations of DA and 5-HT in the spinal cord after procaine (Fig. 2). Thus, we are convinced that the rises of DA and 5-HT levels in the spinal cord after procaine are directly involved with the convulsions and that there is a close correlation between the convulsions by procaine and alterations of DA and 5-HT in the spinal cord.

Chemical transmitters in the descending tracts are not well-known in the spinal cord. However, some studies have shown that NE, DA and 5-HT in the spinal cord are closely correlated with the NE cells in the pons-medulla oblongata (12, 13), the hypothalamus (14), and the nuclei raphe caudalis in the medulla oblongata (13, 15), respectively.

In electrophysiologic studies on experimental animals, local anesthetic-induced convulsions are reported to originate within the amygdala and hippocampus (16, 17). These areas of the limbic system appear to be innervated in part by monoaminergic neurons which utilize NE, DA and 5-HT as neurotransmitters (18).

Consequently, we also believe that the limbic system may be the focus of procaine-induced convulsions and that alterations of monoamines in the spinal cord by procaine...
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