EFFECTS OF BIOGENIC MONOAMINES AND PRECURSORS ON STRESS ULCERS AND SECRETION IN STOMACH

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Abstract—Effects of biogenic monoamines and their precursors on stress ulcers and secretion in stomach were investigated herein. Norepinephrine, 5-HT and their precursors prevented formation of stress ulcer at any level of endogenous monoamines in reserpine- or iproniazid-treated rats. These same agents did not affect the stress-induced decrease in the output of PSB but increased the pH value of gastric juice.

It was reported that experimental ulcers were produced in the cat gastrointestinal tract by small doses of reserpine followed by electrical shock or hypothalamic stimulation and that the mechanism would involve a decrease in central monoamine level (1-6). It was also shown that reserpine or reserpine-like drugs caused gastric hemorrhage by a mechanism which involved release of catecholamines or serotonin (7, 8).

Recently, the present authors have demonstrated that formation of gastric ulcer in rats treated with a combination of reserpine and stress is due to endogenously liberated monoamines and that the decrease in gastric monoamine level prevents formation of the stress ulcer (9). Further studies have shown that the output of injected Pontamine Sky Blue 6BX (PSB) in the gastric juice is decreased by stress, probably due to sustained contractions of gastric mucosal vessels resulted from liberation of monoamines and that there is a reciprocal relationship between the production of stress ulcer and the output of PSB (10).

The present experiments were carried out to determine effects of exogenously administered monoamines and precursors on the production of stress ulcer at various level of endogenous monoamines as well as the effects on the output of PSB in the stomach, in order to correlate the two parameters.

MATERIALS AND METHODS

Stress ulcer: As described previously by Takagi and Okabe (11), male rats of Donryu strain (220-260 g) were immersed into a water bath at 23°C for 7 hr to the level of xiphoid. The animals were then sacrificed, the isolated stomach was inflated with about 10 ml water and placed in 0.5% formalin for 5 min, according to Brodie et al. (12). After washing, the stomach was incised along the greater curvature. Ulcerative changes were scored and expressed as ulcer index, according to grade and number as described by Takagi and Okabe (11).

Determination of output of PSB (13): Male Donryu strain rats (about 250 g) were
fasted overnight. The stomach was exposed under light ether anesthesia and the pylorus ligated. The stomach was washed, then filled with warm saline 2 ml/100 g body wt. through a polyethylene tube. A 5% PSB solution in a volume of 0.2 ml/100 g was injected into the tail vein immediately after washing the stomach. Animals were immersed into a water bath at 23°C for 3 hr to the level of xiphoid. After removal of the stomach, the gastric juice was collected and the volume was determined. The pH value was measured using a glass electrode pH meter.

The amount of PSB in the gastric juice was determined as follows: adjustment of the juice to pH 7.0 with sodium hydroxide, addition of benzenonium chloride solution (40 mg/ml) and adequate amount of chloroform, agitation and centrifugation of the mixture at 3,000 rpm for 10 min and spectrometric reading were done at 620 mµ for PSB in chloroform layer. The value is expressed as µg PSB/100 g of body wt.

Drugs used: Norepinephrine hydrochloride (Sankyo), serotonin creatinine sulfate (5-HT) (Sigma), L-dopa (Taisho), dopamine hydrochloride (Tokyo Kasei), 5-hydroxytryptophan (5-HTP) (Sigma), iproniazid phosphate (Tokyo Kasei), reserpine (Ciba). These drugs were injected intraperitoneally. Except for iproniazid and reserpine which were used in order to modify endogenous monoamine levels, all agents were given 15 min prior to exposure to stress.

RESULTS

Ulcer formation

As shown in Table 1, intraperitoneal injections of norepinephrine 2 mg/kg and 5-HT

| Drug       | Dose (mg/kg) | No. of rats | Ulcer index | Preventive ratio (%) |
|------------|--------------|-------------|-------------|----------------------|
| Control    | 20           | 10          | 19.1±3.5    | 84.6                 |
| L-Dopa     | 20           | 10          | 12.4±2.5    | 31.9                 |
|            | 50           | 15          | 19.1±3.5    | 84.6                 |
|            | 100          | 40          | 20.6±3.6    | 58.7                 |
|            | 5-HT         | 2           | 8.5±3.1*    | 35.4                 |
|            | 3            | 7           | 13.3±3.8    | 58.7                 |
|            | 10           | 9           | 3.6±5.6*    | 82.5                 |
| Control    | 10           | 10          | 19.1±3.5    | 84.6                 |
| Dopamine   | 10           | 10          | 23.5±3.1    | 84.6                 |
|            | 30           | 10          | 15.6±3.3    | 84.6                 |
|            | 100          | 8           | 7.7±5.3*    | 84.6                 |
| Control    | 10           | 10          | 25.7±2.9    | 84.6                 |
| 5-HTP      | 20           | 9           | 10.1±2.6**  | 60.7                 |
|            | 50           | 10          | 11.9±2.4**  | 53.7                 |
|            | 100          | 10          | 7.0±3.3**   | 72.8                 |

Values are means±standard error.

**: p<0.01; *: p<0.05 when compared with controls.
10 mg/kg 15 min prior to the stress caused significant decreases in the ulcer index. Injections of L-dopa and dopamine tended to promote ulcer formation in smaller doses but prevented it in larger doses. Administration of 5-HTP significantly decreased the ulcer index at any dosage level tested.

In animals treated with 5 mg/kg of reserpine 14 hr prior to the stress, the ulcer index decreased to some extent. Administration of norepinephrine 2 mg/kg or L-dopa 50 mg/kg to reserpinized rats 15 min before the stress resulted in 26.7% and 9.3% decreases in the ulcer index, respectively. 5-HT 10 mg/kg and 5-HTP 50 mg/kg also resulted in 16.8% and 37.9% decreases, respectively. Iproniazid 100 mg/kg caused a 50.3% decrease in

| Drugs         | Dose (mg/kg) | No. of rats | Ulcer index | Preventive ratio (%) |
|---------------|--------------|-------------|-------------|----------------------|
| No treatment  |              | 10          | 21.3 ± 3.8  | -32.3                |
| Res.          | 5            | 10          | 16.1 ± 3.7  |                      |
| Res. + Norepi.| 2            | 9           | 11.8 ± 2.7  | 26.7                 |
| Res. + L-Dopa | 50           | 10          | 14.6 ± 2.7  | 9.3                  |
| Res. + 5-HT   | 10           | 10          | 13.4 ± 4.1  | 16.8                 |
| Res. + 5-HTP  | 50           | 10          | 10.0 ± 1.8* | 37.9                 |
| Res. + Ipr.   | 100          | 10          | 8.0 ± 1.3** | 50.3                 |

** Water-immersion stress was given to reserpinized rats 15 min after administration of each drug.
Res.: Reserpine, Norepi.: Norepinephrine, Ipr.: Iproniazid
Values are means ± standard error.
** : p<0.01; *: p<0.05 when compared with “Res.” values.

**Fig. 1. Time course of the incidence of gastric ulcer with iproniazid and water-immersion stress. A 7 hr stress was given at a certain point of time after iproniazid administration. Each point represents the mean of ulceration with vertical lines indicating standard error. Ordinate: ulcer index. Abscissa: time after iproniazid administration.**
the ulcer index. Thus, all agents tested decreased the ulcer index even in reserpinized animals (Table 2).

Fig. 1 demonstrates that the ulcer index tends first to decrease, then to increase following administration of iproniazid 100 mg/kg. In animals pre-treated with iproniazid 16 hr before, injection of L-dopa 50 mg/kg, 5-HT 3 mg/kg or 5-HTP 50 mg/kg decreased the ulcer index (Table 3). Injection of reserpine 1 mg/kg prevented the action of iproniazid.

### Table 3. Effects of drugs on stress ulcer 16 hr after administration of iproniazid (100 mg/kg).

| Drugs       | Dose (mg kg) | No. of rats | Ulcer index | Preventive ratio (%) |
|-------------|--------------|-------------|-------------|----------------------|
| No treatment |              | 10          | 19.8±2.6    | 26.1                 |
| Ipr.        | 100          | 8           | 26.8±4.5    |                       |
| Ipr. + L-Dopa | 100±50      | 5           | 11.0±5.4*   | 59.0                 |
| Ipr. + 5-HT  | 100±3        | 8           | 12.6±2.6**  | 53.0                 |
| Ipr. + 5-HTP | 100±50       | 10          | 9.8±3.6**   | 63.4                 |
| Ipr. + Res. | 100±1        | 10          | 19.7±2.9    | 26.5                 |

Water-immersion stress was given to iproniazid-treated rats 15 min after administration of each drug.

Ipr.: Iproniazid, Res.: Reserpine

**: p<0.01; *: p<0.05 when compared with "Ipr." values.

### Output of PSB and gastric juice

In non-stressed rats, L-dopa 100 mg/kg reduced the volume of gastric juice and output of PSB over a 3 hr observation period, however, the pH value of the juice was increased. 5-HTP 100 mg/kg significantly reduced the output of PSB as well as the volume of gastric juice. Exposure to stress decreased the output of PSB and the volume of gastric juice significantly, as reported earlier (10). Pre-injection of L-dopa or 5-HTP did not signifi-

### Table 4. Effects of L-Dopa and 5-HTP on gastric secretion and output of PSB in stressed and non-stressed rats.

| Treatment | Dose (mg/kg) | No. of rats | Volume (ml) | pH     | PSB output /g/100 g | % change |
|-----------|--------------|-------------|-------------|--------|---------------------|----------|
| Non-stressed |              |             |             |        |                     |          |
| Control   | 4.6±0.2      | 11          | 1.4±0.1     | 16.9±2.1 |
| L-Dopa    | 3.9±0.4      | 12          | 1.9±0.4     | 13.0±2.2 | 23.1                |
| 5-HTP     | 2.8±0.3**    | 12          | 1.6±0.1     | 6.2±0.8** | 63.3                |
| Stressed |              |             |             |        |                     |          |
| Control   | 0.7±0.2      | 11          | 1.6±0.1     | 5.0±1.6  |
| L-Dopa    | 0.3±0.1*     | 12          | 2.5±0.4*    | 5.2±1.4  | -4.0                |
| 5-HTP     | 0.9±0.1      | 12          | 1.8±0.1     | 5.2±1.2  | -4.0                |

Water-immersion stress was given 15 min after administration of L-Dopa and 5-HTP. Values are means±standard error.

**: p<0.01; *: p<0.05 when compared with corresponding controls.
significantly affect the stress-induced decreases in the output of PSB. The pH value of gastric juice increased and the volume of gastric juice was reduced following injection of L-dopa. The results are shown in Table 4.

Table 5 shows the effects of norepinephrine 2 mg/kg and 5-HT 10 mg/kg on the output of PSB and on the volume and pH value of gastric juice. In the non-stressed rats, norepinephrine and 5-HT significantly reduced the output of PSB and the volume of gastric juice. Pre-injection of norepinephrine or 5-HT did not significantly affect the stress-induced decreases in output of PSB and volume of gastric juice. Only the pH value of the gastric juice was significantly increased.

DISCUSSION

There are several reports regarding the effect of biogenic monoamines and precursors on formation of stress ulcer. Exogenously administered norepinephrine and its precursor reduced formation of ulcer due to stress (14-17). 5-HT and the precursor are reported to promote or conversely reduce the ulcer formation (17, 5).

In the present study, formation of stress ulcer was prevented by exogenously administered norepinephrine, 5-HT and its precursor in normal as well as reserpine-or iproniazid-treated rats. A previous report has shown that stress ulcer formation is due to endogenously liberated monoamines and that decrease in the monoamine level by reserpine lowers the incidence at stress ulcer (9). Thus, it can be concluded that exogenously administered monoamines and precursors prevent formation of stress ulcer at any level of endogenous monoamines, whereas endogenously liberated monoamines promote this formation.

Recently, the present authors have shown that exposure of rats to stress results in a reduction of the output of injected PSB in the early stage of reserpinization while an increase is observed in the later stage (10). It is suggested that the output of PSB varies reversely to the amount of released norepinephrine which presumably determines the blood flow and/or capillary permeability of gastric mucosa. In the present experiments, exogen-
Norepinephrine, 5-HT and their precursors reduced the output of PSB and volume of gastric juice in normal rats. Pre-treatment with these agents did not affect the stress-induced decrease in output of PSB. Thus, it is unlikely that reduction of formation of stress ulcer following exogenous administration of monoamines and precursors is due to prevention from stress-induced decrease of blood flow and/or capillary permeability of gastric mucosa. It is assumed that increased pH value of gastric juice may have a causal relationship to the effect of exogenous monoamines and precursors. Further studies are necessary on this respect.

Several investigators attempted to correlate the formation of gastric stress ulcer with the increased turnover rate of central monoamines (18-20). Since the effect of monoamines on formation of stress ulcer did not, however, differ from the effect of precursors which do pass across the blood brain barrier, central monoaminergic mechanisms which may play an important role in prevention of stress ulcer by exogenous monoamines can be excluded.

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