Suppression of Pentylenetetrazol-Induced Seizures by Hydralazine Associated with 5-Hydroxytryptaminergic System in Rat Brain

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Abstract—The effects of hydralazine on the central nervous system were studied in rats. Administration of hydralazine (10 mg/kg, i.p.) transiently, but significantly suppressed the seizures elicited by pentylenetetrazol (PTZ). The suppressive actions were potentiated in the animals pretreated with either reserpine or p-chlorophenylalanine, but not α-methyltyrosine. Methysergide, an antagonist of 5-hydroxytryptamine (5-HT) receptors, could abolish the effect of hydralazine on the tonic component of the seizures, but unlikely that on the clonic one. Although 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels in the brain were both significantly increased after the administration of hydralazine, the increased levels of 5-HIAA reached the peak level earlier than those of 5-HT did. In 5-HT turnover, hydralazine did not change the 5-HT synthesis rate, but the drug inhibited the elimination of 5-HIAA from the brain. The accumulation of 5-HIAA after the inhibition of the acid transport system by probenecid was transiently, but significantly increased in the animals treated with hydralazine. The potency of the suppressive effects of hydralazine on PTZ-induced seizures was in parallel with the rate of 5-HIAA formation in the brain. These results suggest that hydralazine might antagonize the PTZ-induced seizures at least partly by modulating the activation in the central 5-HT-ergic system.

Hydralazine has been clinically used as a potent antihypertensive drug for three decades. However, its effects on the central nervous system have been rarely elucidated since it was concluded that central vasomotor effects negligibly participated in the hypotensive action of the drug (1).

We recently reported that hydralazine at a dose of 10 mg/kg not only potentiated the hypnotic effects of barbiturates which were not associated with the metabolisms in the liver (2), but also suppressed the seizures elicited by pentylenetetrazol (PTZ), but not those by picrotoxin (3). In contrast, it was also found that hydralazine at higher doses (over 40 mg/kg) caused seizures whose threshold was decreased by the combination with isoniazid (4) and that hydralazine at a dose of 40 mg/kg potentiated picrotoxin-induced seizures, while it slightly strengthened PTZ-induced ones (3). These evidences indicated that although hydralazine possessed significant effects on the central nervous system, its effects at higher doses would be different from those at lower doses.

On the other hand, it is well established that the threshold of PTZ-induced seizures can be altered by modifying central monoaminergic systems (5–9), and central excitation derived from picrotoxin and hydrazine derivatives such as isoniazid originated from the indirect antagonism of 7-
aminobutyric acid (GABA) receptor (10) and the decrement of the synaptic GABA level (11, 12), respectively. It was, therefore, considered that the excitatory effects of hydralazine at toxic doses might be closely associated with the changes in the central GABA-ergic system (3, 4) and that the suppressive effects of the drug at lower doses might be related to central monoaminergic systems (3).

It was thought that the evidence indicated that hydralazine at the lower doses would be applicable for clinical use, although high ones have toxic effects. Therefore, we performed the further study of the relationship between the suppressive effects of hydralazine against PTZ-induced seizures and central monoaminergic systems.

Materials and Methods

Animals and drug treatments: All experiments were performed during the period from 10:00 to 16:00 using male Wistar rats weighing 180 to 220 g. The animals were housed at a constant temperature (24±1°C) on a 12 hr light-dark cycle (on, 7:00 to 19:00), and they were allowed access to food and water ad libitum.

The following drugs were administered intraperitoneally before PTZ administration at the time interval indicated: Hydralazine hydrochloride (Sigma), 10 mg/kg, 1 hr; reserpine (Tokyo Kasei), 2 mg/kg, 18 hr; p-chlorophenylalanine (PCPA, Sigma), 300 mg/kg, 48 hr; α-methyltyrosine methylster hydrochloride (Nakarai), 200 mg/kg, 4 hr; methysergide hydrogenmaleinate (Sandoz), 5 mg/kg, 30 min. Reserpine was dissolved in 0.3 M sucrose solution with a few drops of glacial acetic acid. PCPA was suspended in distilled water with a few drops of Tween 80. Other drugs were dissolved in isotonic saline immediately before use.

Evaluation of the behavioral changes after PTZ administration: Behavioral changes after PTZ administration were scored according to the index of seizure severity (Table 1), as previously reported (3, 13). The observation of the animals was continuously carried out for 1 hr after PTZ. PTZ was injected subcutaneously at a dose of 100 mg/kg, except in reserpinized animals, because it is well-known that reserpine increases the seizure susceptibility (14, 15). In order to obtain the comparable final seizure severity score in reserpinized animals without hydralazine to that in intact ones, 80 mg/kg of PTZ was used in reserpinized animals.

Determinations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels in the brain: Rats were killed by decapitation, and the brains were rapidly removed, frozen on dry ice and stored at −70°C until analyzed, usually within 4 days after the death. Simultaneous determinations of 5-HT and 5-HIAA levels were carried out according to the fluorometric method of Peret-Cruet et al. (16) with minor modification by Fukumori et al. (17).

Determinations of 5-HT synthesis rate and 5-HIAA elimination rate in the brain: Determinations of the rates of 5-HT synthesis and 5-HIAA elimination were performed essentially according to the methods of Tozer et al. (18) and Neff and Tozer (19) as follows. Rat brains were removed at various times after the administration of pargyline hydrochloride (Nakarai; 75 mg/kg, i.p.); and 5-HT and 5-HIAA levels were assayed as described above. Pargyline was injected 30 min after hydralazine (10 mg/kg). 5-HT synthesis rate was determined by calculating the linear regression of 5-HT accumulation after pargyline. The elimination rate of 5-HIAA was calculated by multiplying the initial level of 5-HIAA by the rate constant of 5-HIAA efflux from the brain. The rate constant was determined from the slope of the exponential decline of 5-HIAA levels after pargyline.

Determination of the accumulation of brain 5-HIAA: The accumulation of 5-HIAA in the brain was determined by the method of Neff et al. (20). Rats were decapitated at various times after the injection of probenecid (Sigma; 200 mg/kg, i.p.) which inhibits the acid transport system in the brain, and brain 5-HIAA levels were determined as described above. Probenecid was injected intraperitoneally 30 min after hydralazine (10 mg/kg).

Statistical analysis: Statistical analysis was performed using Student's t-test.
Results

Figure 1A shows the effects of hydralazine on PTZ-induced seizures in intact rats. The seizure severity scores in the control group were gradually increased in parallel with time after PTZ administration. On the other hand, the scores in hydralazine-treated animals were significantly suppressed until 30 min after PTZ; thereafter, the suppression gradually disappeared, and the seizure severity scores were comparable to those of the control at the end of the observation period (60 min after PTZ, corresponded to 120 min after hydralazine) as previously reported (3). Graphs B, C and D in Fig. 1 show the effects of pretreatment with reserpine, PCPA and α-methyltyrosine, respectively, on the suppressive effects of hydralazine against PTZ-induced seizures. It is well known that PCPA and α-methyltyrosine decrease 5-HT and catecholamines in the brain, respectively, by inhibiting the rate limiting enzyme in each synthesis (21, 22), and reserpine depletes both monoamines. As shown in Fig. 1B, it seemed that the actions of hydralazine were potentiated by the pretreatment with reserpine because the suppression was continued until 60 min after PTZ, differing from that in intact animals. It was also observed that in hydralazine-treated animals, it was difficult to cause the tonic component of the seizures (score 4 as shown in Table 1). In PCPA-treated rats, the severity scores of the control group increased more sharply than those of the intact control, which is consistent with the fact that PCPA exacerbates the PTZ-induced seizures.

Fig. 1. Effects of pretreatment with reserpine, PCPA and α-methyltyrosine on the suppressive effects of hydralazine against PTZ-induced seizures. (A) non-pretreated, (B) reserpinized, (C) PCPA-pretreated, (D) α-methyltyrosine-pretreated. Reserpine (2 mg/kg), PCPA (300 mg/kg) and α-methyltyrosine (200 mg/kg) were injected intraperitoneally 18, 48 and 4 hr prior to the administration of PTZ, respectively. Data represent the mean±S.E.M. of the seizure severity scores of 8 to 10 rats. ( —○—) control; ( —●—) hydralazine, 10 mg/kg, i.p. *, ** and *** donate significant (P<0.05, P<0.01 and P<0.001, respectively) differences from the corresponding control.
seizures in rats (7, 8). However, the final scores of the control in PCPA-treated animals were only slightly higher than those in intact ones. It appeared in Fig. 1C that the suppressive effects were relatively strengthened by the pretreatment with PCPA since the scores were significantly reduced even at 60 min after PTZ, although the pattern of the behavioral changes essentially resembled that in the intact group. In contrast, α-methyltyrosine almost failed to change the effects of hydralazine (Fig. 1D).

Table 2 represents the effects of methysergide, an antagonist of 5-HT receptors (23), on the actions of hydralazine on the time to onset of clonic and tonic seizures induced by PTZ. Hydralazine alone administration prolonged the time to onset of both seizure components. When methysergide was combined, the effect of hydralazine on tonic seizure, but not clonic one, was abolished.

From the biochemical analysis, both 5-HT and 5-HIAA levels in the brain were found to be significantly increased during the period from 60 to 120 min after hydralazine administration, which corresponded to the observation period of the behavioral changes after PTZ (Fig. 2). 5-HT level in the brain reached the peak 90 min after hydralazine. In contrast, brain 5-HIAA had already achieved the peak level 60 min after hydralazine (Fig. 2). Table 3 shows the effects of hydralazine on 5-HT turnover rate in rat brain. Although hydralazine did not change the 5-HT synthesis rate, 5-HIAA elimination was decreased to 60% of the control. On the other hand, accumulated levels of brain 5-HIAA were expressed as a function of time after the injection of probenecid (Fig. 3). The initial levels of 5-HIAA were not different between the control and hydralazine-treated animals. The accumulation of 5-HIAA in the brain was significantly enhanced by the treatment with hydralazine 30 and 60 min after probenecid, which corresponded to the time of 0 and 30 min after PTZ, respectively. However, the accumulated 5-HIAA levels in rat brain treated with
Table 3. Effects of hydralazine on 5-HT turnover rate in rat brain

| Treatment          | Control          | Hydralazine      |
|--------------------|------------------|------------------|
| Initial level of 5-HT (µg g brain⁻¹) | 0.6757±0.0254     | 0.7359±0.0172    |
| 5-HT synthesis rate (µg g brain⁻¹·hr⁻¹) | 0.2950           | 0.2899           |
| (nmole·g brain⁻¹·hr⁻¹) | 1.6741           | 1.6452           |
| Rate constant of 5-HIAA efflux (hr⁻¹) | 0.7446           | 0.4700           |
| Initial level of 5-HIAA (µg g brain⁻¹) | 0.3985±0.0143     | 0.3789±0.0143    |
| 5-HIAA elimination rate (µg/g brain⁻¹·hr⁻¹) | 0.2967           | 0.1781           |
| (nmole·g brain⁻¹·hr⁻¹) | 1.5518           | 0.9315           |

Fig. 2. Time course study on brain 5-HT and 5-HIAA levels after hydralazine (10 mg/kg, i.p.). Initial levels of 5-HT and 5-HIAA in the brain were 0.564±0.019 and 0.306±0.009 µg/g brain, respectively. (—•—) 5-HT, (—○—) 5-HIAA. 8 animals were used for the determinations on each point. Statistically significant differences from the initial levels: *P<0.05, **P<0.01, ***P<0.001.

Fig. 3. Effect of hydralazine on 5-HIAA accumulation in the brain after probenecid. Probenecid (200 mg/kg, i.p.) was injected 30 min after hydralazine (10 mg/kg, i.p.). Each point represents the mean ± S.E.M. of 8 to 10 animals. (—•—) control, (—○—) hydralazine. Statistically significant difference from the control: *P<0.02, **P<0.01.

Discussion

In the present study, we first did the pretreatment with reserpine, PCPA and α-methyltyrosine in order to examine the suppressive effects of hydralazine against PTZ-induced seizures in rats with lower levels of 5-HT and/or catecholamines. It is well-documented that PCPA and α-methyltyrosine interfere with the syntheses of 5-HT and catecholamines in the brain, respectively (21, 22), and reserpine depletes both types of monoamines. As previously reported (3), hydralazine, when administered...
to intact rats, caused transient and significant suppression of the seizures elicited by PTZ (Fig. 1A). These anticonvulsant actions could be potentiated by the pretreatment of rats with either reserpine or PCPA, while α-methyltyrosine failed to alter them (Fig. 1). These results led to the hypothesis that the central 5-HT-ergic system might be involved in the anticonvulsant effects of hydralazine. To examine this possibility, rats were challenged with hydralazine in combination with methysergide, an antagonist of 5-HT receptors. As shown in Table 2, methysergide had no effects on the suppression of the clonic component of PTZ-induced seizures by hydralazine, but completely abolished the effect of hydralazine on the tonic component of the seizures.

De la Torre and his coworkers (7, 8) reported that PTZ-induced seizures were exacerbated by PCPA; and conversely they were alleviated by 5-hydroxytryptophan, a precursor of 5-HT. In addition, Bhattachrya and Sanyal (24) demonstrated that the incidence of seizures after PTZ administration was significantly reduced by prostaglandin E\(_1\), which enhances 5-HT turnover (25, 26). Kilian and Frey (9) suggested that although 5-HT played a role mediating both clonic and tonic components of PTZ-induced seizures, it might be more crucially involved in the latter. Moreover, Minegishi et al. (27) found that tryptophol, a dehydroxylated analogue of a 5-HT metabolite, prolonged the time to onset of tonic seizure after PTZ, but not the clonic one and suggested that it has a possible role in the modulation of the seizure pattern by the central 5-HT-ergic system. Taking these reports and the data in this study into account, it is likely that the activated 5-HT-ergic system may at least in part contribute to the suppression of PTZ-induced seizures by hydralazine.

From this viewpoint, we examined the effects of hydralazine on the central 5-HT system by means of determining both brain levels of 5-HT and its major metabolite, 5-HIAA. 5-HT and 5-HIAA levels in the brain were both significantly increased during the period from 60 to 120 min after hydralazine which corresponded to the observation period after PTZ (Fig. 2). It also appeared that 5-HIAA reached the peak level earlier than 5-HT did. The results of the measurement of 5-HT turnover indicated that hydralazine did not alter the central 5-HT synthesis rate and that it delayed 5-HIAA elimination from the brain (Table 3). One may, therefore, consider that the increment of brain 5-HT levels after hydralazine is due to the inhibition of monoamine oxidase (MAO) by the drug. In fact, it was reported that hydralazine (28) and its metabolite (29) could inhibit the enzyme in the brain. One may further expect that the increased levels of 5-HIAA after hydralazine can be ascribed to the delayed elimination of 5-HIAA. If so, the production of 5-HIAA, the predominant metabolite of 5-HT, should be reduced. Contrary to this expectation, the accumulated 5-HIAA concentrations by probenecid, indicating its production rate from 5-HT (20), were significantly increased 30 and 60 min after probenecid in the hydralazine-treated group (Fig. 3). However, it seemed that the production of 5-HIAA was relatively reduced during the period from 60 to 90 min after probenecid because 5-HIAA level in the hydralazine-treated group was comparable to that of the control 90 min after probenecid (Fig. 3). These findings indicate that brain MAO may not be inhibited at least 30 to 90 min after hydralazine, and then, its activity may be interfered with to some extent by the time of 120 min after the drug. They also suggest that the increased levels of 5-HIAA by hydralazine administration may be due not only to the delay on the 5-HIAA elimination but also to the enhancement of the formation from 5-HT. Here, referring the behavioral changes after PTZ (Fig. 1A) to the alterations of the accumulated levels of 5-HIAA after probenecid (Fig. 3), keeping in mind the fact that the observation period after PTZ administration corresponded to 30 to 90 min after probenecid, it is notable that when 5-HIAA production was accelerated, the increment of the seizure severity scores was suppressed and vice versa.

On the basis of these findings, it is concluded that hydralazine may antagonize PTZ-induced seizures at least in part through the modulation of the central 5-HT-ergic system, where it might probably facilitate
5-HT release from the synaptic vesicles.

On the other hand, we could not obtain evidence to explain the discrepancy that both 5-HT and the production of 5-HIAA were increased by hydralazine administration despite the fact that 5-HT synthesis rate was not altered. Fukumori et al. (17) suggested that the increase of the neutral metabolite of 5-HT, 5-hydroxyindoleacetaldehyde (5-HIAAld), could modulate 5-HT turnover in the brain. Furthermore, Minegishi et al. (30) suggested the product inhibition of MAO by 5-HIAAld from the result that when it was increased, 5-HT level in the brain was also increased in spite of no change in 5-HT synthesis rate. A hypothesis, therefore, would be raised that an autoregulatory mechanism(s) of the 5-HT system such as feedback regulation might go into operation by the enhancement of 5-HT metabolism, since reduction of 5-HIAA formation was observed after its transient acceleration as shown in Fig. 3.

Further studies are being undertaken to clarify the potentiating abilities of reserpine and PCPA on the suppressive effects of hydralazine. Furthermore, other studies in addition to ones on the central monoaminergic system are needed to understand the suppression induced by hydralazine in the central nervous system because Abdul-Ghani et al. (31) reported the attenuation of PTZ-induced seizures by the antagonists of glutamate, a putative neurotransmitter.

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