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Benzodiazepines Increase Tonic Component of Postdecapitation Convulsions in Mice

TAKAHIDE HARA, ITSUKO USHIJIMA, SHUUHEI KAWAZAWA, YASUSHI MIZUKI AND MICHIO YAMADA

Department of Neuropsychiatry, Yamaguchi University School of Medicine 1144 Kogushi Ube City, 755 Japan

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HARA, T., I. USHIJIMA, S. KAWAZAWA, Y. MIZUKI AND M. YAMADA. *Benzodiazepines increase tonic component of postdecapitation convulsions in mice.* PHARMACOL BIOCHEM BEHAV **30**(4) 1001–1006, 1988.—The effects of benzodiazepines (BDZs) and GABA system on tonic and clonic component of postdecapitation convulsion (PDC) were studied in mice. Mice decapitated at the occipito-cervical junction, exerted biphasic convulsions, i.e., initially tonic and subsequently clonic convulsions. BDZs such as diazepam or clonazepam increased tonic and clonic components of PDC. These effects were not antagonized by Ro 15-1788, a benzodiazepine receptor antagonist. The increased tonic component was antagonized by the GABA receptor antagonists, bicuculline and picrotoxin, whereas the clonic component was augmented by them. Aminooxyacetic acid, which increases the endogenous GABA content by inhibiting the GABA-transaminase, increased the tonic component significantly; this increase was antagonized by both bicuculline and picrotoxin. Muscimol, a GABA agonist, however did not affect the tonic components but rather augmented the clonic component. Bicuculline and picrotoxin did not antagonize this effect of muscimol. These results indicate that endogenous GABA may play a crucial role in mediating the tonic component of PDC and the facilitation of this component by BDZs may also be due to the activation of GABA in the spinal cord. Furthermore, the mechanisms of the tonic component may be different from that of the clonic component.

Postdecapitation convulsions Tonic Clonic Benzodiazepine GABA AOAA Mice

TRANSECTION of the cervical spine produces violent generalized convulsions in the rat, mouse and rabbit [6]. The mouse displays vigorous tail lifting followed by tonic extension of the hind limbs and subsequently, their violent, coordinated flexions and extensions. This phenomenon is called the postdecapitation reflex or postdecapitation convulsions (PDC), which include tonic and clonic components. PDC may be a model of spinal seizures because the spinal cord is the highest center of the motor system in the beheaded animals and the likelihood of epileptiform activation is greatest at this point and progressively decreases towards the periphery of the involved area [11].

The observation that an injection of the direct-acting 5-hydroxytryptamine (5-HT) receptor agonist, 5-methoxy-n, n-dimethyltryptamine, in rats prolongs the latency to onset and the duration of clonic PDCs indicates that inhibitory 5-HT receptors are involved in the initiation of PDC [2] which are totally dependent on an intact noradrenergic system in the spinal cord [15]. Although it is obvious that there are tonic and clonic components to this phenomenon in the mouse, previous reports have been only concerned with the clonic phase [7, 10, 12, 15]. If PDC originates at the spinal cord, it is of interest to study the effect of benzodiazepines (BDZs) on PDC because diazepam has been shown to augment spinal myoclonus in photosensitive and nonphotosensitive baboons [17]. It is generally accepted that BDZs exert the majority of their pharmacological effects by enhancing GABA neurotransmission and that [^{3}H] BDZ binding is enhanced by the presence of GABA [16]. Therefore, we pursued a series of experiments aimed at examining the effect of BDZs and GABAergic drugs on the tonic and clonic components of PDC in mice.

METHOD

Animals

Healthy male albino mice of the ddY strain, weighing 28–35 g at 6 weeks old were obtained from Kyudo Animal Laboratory (Kumamoto, Japan) and housed ten to fifteen per cage for one week before the experiment in an animal room with light-dark cycle of 12-12 hours. Commercial food (MF, Oriental Yeast Ltd.) and tap water were available ad lib. The animals fasted three hours before the administration of drugs. All experiments were performed at an environmental temperature of 23±1°C.

Drugs

Diazepam provided by Takeda (Osaka, Japan), clon-

TABLE 1

EFFECTS OF DIAZEPAM AND CLONAZEPAM ON THE TONIC AND THE CLONIC COMPONENTS OF THE POSTDECAPITATION CONVULSIONS AT VARIOUS DOSES

Dose (mg/kg)		Onset Sec	Tonic Sec	Clonic Sec	Number	Frequency
Control	n=33	2.4 ± 0.1	3.6 ± 0.4	13.6 ± 0.5	17.6 ± 0.6	1.31 ± 0.03
Diazepam (2.5)	n=10	2.5 ± 0.1	4.8 ± 0.6	13.8 ± 1.2	19.0 ± 1.5	1.40 ± 0.06
Diazepam (5)	n=15	2.5 ± 0.2	5.0 ± 0.5*	14.4 ± 0.8	21.3 ± 1.1†	1.50 ± 0.06†
Diazepam (10)	n=25	2.3 ± 0.1	5.5 ± 0.6†	15.3 ± 0.7	21.8 ± 0.9‡	1.45 ± 0.04†
Diazepam (20)	n=10	2.3 ± 0.1	7.3 ± 0.4‡	12.4 ± 1.0	17.8 ± 1.6	1.44 ± 0.06*
Clonazepam (0.1)	n= 5	2.2 ± 0.1	2.7 ± 0.8	14.5 ± 1.9	18.6 ± 1.5	1.33 ± 0.11
Clonazepam (0.2)	n= 4	2.6 ± 0.3	3.6 ± 1.0	14.7 ± 0.7	22.0 ± 0.7*	1.52 ± 0.11*
Clonazepam (0.5)	n=10	2.2 ± 0.1	7.1 ± 0.5‡	12.9 ± 0.5	22.0 ± 0.6‡	1.72 ± 0.06‡
Clonazepam (1)	n=10	2.0 ± 0.2	6.9 ± 0.6‡	15.2 ± 1.0	20.7 ± 1.6*	1.38 ± 0.08*

Results are shown as mean ± SEM. Animals were decapitated 30 min after administration of the drugs. Tonic: the duration of the tonic component (sec); clonic: the duration of the clonic phase (sec); number: the number of clonic convulsions; frequency: the frequency of clonic convulsions. *$p<0.05$, †$p<0.01$, ‡$p<0.001$, significant difference from control, determined by analysis of variance and subsequent t-test or Welch's method.

TABLE 2

EFFECTS OF TIME AFTER ADMINISTRATION OF DIAZEPAM (10 mg/kg) ON TONIC AND CLONIC COMPONENTS OF THE POSTDECAPITATION CONVULSIONS

Time		Onset Sec	Tonic Sec	Clonic Sec	Number	Frequency
Control	n=33	2.4 ± 0.1	3.6 ± 0.4	13.6 ± 0.5	17.6 ± 0.6	1.31 ± 0.03
15 min	n=10	2.2 ± 0.2	4.7 ± 0.5	15.9 ± 0.6*	21.3 ± 0.7†	1.36 ± 0.08
30 min	n=25	2.3 ± 0.1	5.5 ± 0.6†	15.3 ± 0.7	21.8 ± 0.9‡	1.45 ± 0.04
60 min	n=10	2.0 ± 0.2	5.3 ± 0.9	16.7 ± 1.1†	22.6 ± 1.9*	1.35 ± 0.05
120 min	n=10	1.9 ± 0.2	7.2 ± 0.5‡	13.1 ± 1.0	18.3 ± 1.2	1.42 ± 0.06

Results are shown as mean ± SEM. Time: the time between administration of the drug and the decapitation of the animal; tonic: the duration of the tonic component (sec); clonic: the duration of the clonic component (sec); number: the number of the clonic convulsions; frequency: the frequency of the clonic convulsions. *$p<0.05$, †$p<0.01$, ‡$p<0.001$, significant difference from control, determined by analysis of variance and subsequent t-test or Welch's method.

azepam and Ro 15-1788 provided by Hoffmann La Roche (Basel, Switzerland) and aminooxyacetic acid (AOAA) from Sigma (St. Louis, MO) were dissolved in saline with 3% Tween 80. Bicuculline and picrotoxin obtained from Sigma (St. Louis, MO) were dissolved in saline with 3% Tween 80 and a drop of 0.2 N HCl. Muscimol from Sigma (St. Louis, MO) was dissolved in saline. Drugs were administered intraperitoneally in saline solution or suspension with 3% Tween 80 such that the volume injected was 0.1 ml/10 g body weight. Controls were given an equal volume of saline or vehicle. Antagonists were injected 40 min prior to decapitation. AOAA was injected 6 hr before decapitation.

Experimental Procedure

Thirty minutes after the administration of an agonist, mice were decapitated by scissors at the occipito-cervical junction and the body immediately placed feet downwards on a rubber pad in a sink. The latency of onset of a tonic convulsion and the duration of tonic and clonic convulsions were recorded by means of two digital stopwatches (CITIZEN, LSW 9102) which measured the lap time and are graduated in hundredths of a second. The stopwatch in the left hand was started at the time of decapitation and that in the right hand started at the onset of tonic convulsions which was defined as the start of tail lifting. The lap time was recorded at the onset of tonic convulsions by the stopwatch in the left hand; this time was taken as the latency of onset. The stopwatch in the right hand was stopped at the start of biclonal kicks. This time was recorded as the duration of tonic convulsions. The stopwatch in the left hand was stopped at the end of the biclonal kicks. The difference between this time and the sum of the latency and the duration of the tonic phase was taken as the duration of the clonic phase. The number of volleys of clonic convulsions was counted by another experimenter with a digital counter. All observations were made by the same individuals to avoid personal differences in recordings. To eliminate experimenter bias the individual responsible for administration and group allocation was not involved in the time-keeping. Data were eval-

TABLE 3

EFFECTS OF BDZs AND GABA AGONISTS ON TONIC AND CLONIC COMPONENTS OF THE POSTDECAPITATION CONVULSIONS

Dose (mg/kg)		Onset Sec	Tonic Sec	Clonic Sec	Number	Frequency
Control	n=33	2.4 ± 0.1	3.6 ± 0.4	13.6 ± 0.5	17.6 ± 0.6	1.31 ± 0.03
Diazepam (10)	n=25	2.3 ± 0.1	5.5 ± 0.6†	15.3 ± 0.7	21.8 ± 0.9‡	1.45 ± 0.04†
Clonazepam (1)	n=10	2.0 ± 0.2	6.9 ± 0.6‡	15.2 ± 1.0	20.7 ± 1.6*	1.38 ± 0.08*
Muscimol (2)	n=10	3.1 ± 0.4	2.6 ± 0.6	15.3 ± 1.0	23.9 ± 1.3‡	1.59 ± 0.09*
AOAA (30)	n=10	1.9 ± 0.2*	6.8 ± 0.9‡	15.3 ± 1.3	20.5 ± 1.0*	1.56 ± 0.05‡
Baclofen (8)	n=20	4.1 ± 0.2‡	4.5 ± 0.6	15.6 ± 1.0	20.6 ± 1.3†	1.36 ± 0.07

Results are shown as mean ± SEM. Tonic: the duration of the tonic component (sec); clonic: the duration of the clonic phase (sec); number: the number of the clonic convulsions; frequency: the frequency of the clonic convulsions. *$p<0.05$, †$p<0.01$, ‡$p<0.001$, significant difference from control, determined by analysis of variance and subsequent t-test or Welch's method.

uated by analysis of variance followed by test of Welch's method.

RESULTS

Decapitation of mice precipitated tonic convulsions followed by clonic convulsions in 100% of the trials. In the control group, latencies to the onset of tonic convulsions was 2.4±0.1 sec (mean±SEM). The duration of the tonic phase was 3.6±0.4 sec. The duration of clonic phase was 13.6±0.5 sec. The number of volleys of clonic convulsions was 17.6±0.6 and the frequency of clonic convulsions, i.e., the ratio of the number of volleys of clonic convulsions to the duration of clonic phase was 1.31±0.03 (Table 1). These parameters were all normally distributed.

Table 1 shows the effects of diazepam and clonazepam on tonic and clonic components of PDC at various doses. Doses of 10 mg/kg diazepam and 1 mg/kg clonazepam were most effective in prolonging the duration of both tonic and clonic components. These doses were active for mice which became calm and ataxic within 15 min of administration of drugs. Table 2 shows the effect of time after administration of diazepam (10 mg/kg) on tonic and clonic components of PDC. Times of thirty minutes or sixty minutes between administration of the drug and the decapitation of the animal appeared to be most effective in prolonging the duration of both tonic and clonic component of PDC.

Diazepam (10 mg/kg) prolonged the period of tonic convulsions to 5.5±0.6 sec ($p<0.01$) and substantially increased the duration of clonic convulsions (Table 1). Furthermore, it increased the number of volleys of clonic convulsions and the frequency of clonic convulsions to 21.8±0.9 and 1.45±0.04, respectively ($p<0.001$, $p<0.01$) (Table 1). Clonazepam (1 mg/kg), which is a more potent anticonvulsant than diazepam on a mg/mg basis, also increased the duration of the tonic phase and the number of the clonic volleys to 6.9±0.6 sec and 20.7±1.6, respectively ($p<0.001$, $p<0.05$). At 1 mg/kg, it also increased the frequency of clonic convulsions to 1.38±0.08 ($p<0.05$) and substantially increased the duration of clonic convulsions (Table 1).

In the next set of experiments we attempted to reverse the effects of BDZs on the tonic and clonic components of PDC by administering the benzodiazepine antagonist Ro 15-1788 and the GABA antagonists, bicuculline and picrotoxin (Table 4). The Ro 15-1788 treatment, however, did not eliminate the effects of BDZs on the convulsions. On the other hand, bicuculline (4 mg/kg) treatment significantly reduced the effects of BDZs on the duration of tonic convulsions ($p<0.05$ for diazepam and $p<0.01$ for clonazepam) and the frequency of clonic convulsions ($p<0.01$ for diazepam and $p<0.05$ for clonazepam). It did not eliminate the effect of BDZs on the duration of clonic convulsions or the number of volleys of clonic convulsions; rather, it augmented them (Table 4). Bicuculline (4 mg/kg) itself did not decrease the duration of the tonic component of PDC but rather prolonged it ($p<0.05$). Combined treatment with picrotoxin then induced the prolongation of the latency to the onset of tonic convulsions ($p<0.05$ for diazepam and $p<0.01$ for clonazepam) and a decrease in the duration of the tonic component of PDC ($p<0.05$ for clonazepam) and the frequency of clonic convulsions ($p<0.001$ for diazepam and $p<0.1$ for clonazepam). It increased the effect of BDZs on the duration of the clonic phase ($p<0.05$ for diazepam) (Table 4). A dose of 3 mg/kg of picrotoxin alone shortened the duration of tonic convulsions and increased the duration of clonic convulsions and the number of volleys of clonic convulsions.

Muscimol (2 mg/kg), a GABA-receptor agonist, did not affect the tonic phase (Table 5). It increased the number of volleys of clonic convulsions and the frequency of clonic convulsions to 23.9±0.8 sec ($p<0.001$) and 1.59±0.09 ($p<0.05$), respectively. Bicuculline antagonized the effect of muscimol on the number and the frequency of clonic convulsions ($p<0.05$ and $p<0.01$, respectively) (Table 5). The tonic phase was significantly shortened with a combined treatment of bicuculline and muscimol ($p<0.001$).

AOAA (30 mg/kg) treatment significantly shortened the latency of onset ($p<0.05$) and increased the duration of tonic convulsions, the number of volleys of clonic convulsions and the frequency of clonic convulsions to 6.8±0.9 ($p<0.001$), 20.5±1.0 ($p<0.05$) and 1.56±0.05 ($p<0.001$), respectively, Table 3). Bicuculline antagonized the effect of AOAA on the duration of the tonic phase, the number of volleys and clonic convulsions and the frequency of clonic convulsions ($p<0.1$, $p<0.05$ and $p<0.001$, respectively). It did not antagonize the effect of AOAA on the latency and the duration of the clonic phase (Table 5). Picrotoxin antagonized the effect of AOAA on the latency, the duration of the tonic phase and the frequency of clonic convulsions ($p<0.05$, $p<0.05$ and $p<0.001$, respectively) (Table 5).

TABLE 4

ANTAGONISM FOR THE EFFECT OF BDZs ON THE PDC BY Ro 15-1788, BICUCULLINE AND PICROTOXIN

Dose (mg/kg)		Onset Sec	Tonic Sec	Clonic Sec	Number	Frequency
Control	n=33	2.4 ± 0.1	3.6 ± 0.4	13.6 ± 0.5	17.6 ± 0.6	1.31 ± 0.03
Diazepam (10)	n=25	2.3 ± 0.1	5.5 ± 0.6†	15.3 ± 0.7	21.8 ± 0.9‡	1.45 ± 0.04†
Clonazepam (1)	n=10	2.0 ± 0.2	6.9 ± 0.6‡	15.2 ± 1.0	20.7 ± 1.6*	1.38 ± 0.08
Ro 15-1788 (15) + diazepam (10)	n=10	2.0 ± 0.2	5.6 ± 0.7*	15.4 ± 0.8	20.9 ± 0.9*	1.37 ± 0.06
Ro 15-1788 (10) + clonazepam (1)	n=10	2.7 ± 0.2**	6.0 ± 0.6†	13.3 ± 1.0	18.8 ± 1.2	1.42 ± 0.04
Bicuculline (4) + diazepam (10)	n=10	2.1 ± 0.1	3.2 ± 0.8§	19.5 ± 1.7‡	24.0 ± 1.5‡	1.25 ± 0.04†
Picrotoxin (3) + diazepam (10)	n=10	2.9 ± 0.2*§	4.2 ± 0.8	19.4 ± 1.5‡§	19.4 ± 1.1	1.03 ± 0.05‡#
Bicuculline (4) + clonazepam (1)	n=10	2.0 ± 0.2	3.9 ± 0.6††	17.2 ± 1.1†	20.0 ± 1.1*	1.17 ± 0.05*.**
Picrotoxin (2) + clonazepam (1)	n=10	2.8 ± 0.2††	5.1 ± 0.4*.**	17.3 ± 0.9†	20.5 ± 1.5	1.19 ± 0.06*
Ro 15-1788 (10)	n=10	2.0 ± 0.2	4.8 ± 0.7	11.2 ± 1.3*	17.3 ± 1.2	1.62 ± 0.09‡
Bicuculline (4)	n=10	2.4 ± 0.2	5.2 ± 0.6*	11.0 ± 0.7*	15.1 ± 0.9	1.38 ± 0.07
Picrotoxin (2)	n=10	2.4 ± 0.2	4.4 ± 0.6	14.0 ± 0.6	18.0 ± 0.6	1.29 ± 0.04
Picrotoxin (3)	n=10	2.3 ± 0.1	1.9 ± 0.5*	16.6 ± 0.8†	21.0 ± 1.0*	1.27 ± 0.04

Results are shown as mean ± SEM. Tonic: the duration of the tonic component (sec); clonic: the duration of the clonic phase (sec); number: the number of clonic convulsions; frequency: the frequency of clonic convulsions. *$p<0.05$, †$p<0.01$, ‡$p<0.001$, significant difference from control; §$p<0.05$, ¶$p<0.01$, #$p<0.001$, significant difference from diazepam; **$p<0.05$, ††$p<0.01$, significant difference from clonazepam, determined by analysis of variance and subsequent t-test or Welch's method.

Baclofen (β-(p-chlorophenyl)-GABA) (8 mg/kg) significantly increased the latency to the onset of tonic convulsions to 4.1±0.2 sec ($p<0.001$) and 20.6±1.3 ($p<0.01$) respectively (Table 3).

DISCUSSION

Benzodiazepines (BDZs) such as diazepam or clonazepam increased the duration of the tonic and clonic components of postdecapitation convulsions (PDC) and also the number and the frequency of clonic convulsions of PDC in mice. They facilitated PDC, whereas they are anticonvulsants in epilepsy [8]. Thus, the mechanisms of PDC would appear to be different than those for the convulsions originating from the cortices.

Diazepam neither reduces nor obliterates spinal myoclonus in humans [9] and it has been shown to augment spinal myoclonus in photosensitive and nonphotosensitive baboons [17]. Thus, the mechanisms in PDC could be similar to those of spinal myoclonus rather than epilepsies with a cortical origin.

The stimulatory effects of BDZs on PDC were not antagonized by Ro 15-1788, a benzodiazepine-receptor antagonist. BDZs were active at these doses because mice became calm and ataxic within 15 min of administration of drugs. It seems that the BDZ receptor itself does not mediate the phenomenon but rather modulates it. It is generally accepted that BDZs exert the majority of their pharmacological effects by enhancing GABA neurotransmission and that [^{3}H] BDZ binding is enhanced by the presence of GABA [16]. We therefore attempted to reverse the effects of BDZs on PDC times by administering the GABA receptor antagonists, bicuculline or pricotoxin.

Bicuculline treatment significantly eliminated the effect of BDZs on the duration of tonic convulsions and the frequency of clonic convulsions (Table 4). It did not eliminate the effect of BDZs on the duration of clonic convulsions or the number of volleys of clonic convulsions, but rather augmented them. In addition, bicuculline itself did not reduce the duration of the tonic component of PDC but rather prolonged it. Furthermore, it decreased the duration of the clonic component of PDC. Thus, the apparent blockade of the effect of BDZs on the tonic component seen after bicuculline could not be the result of combined drug effects. The apparent augmentation of the clonic component of PDC by combination of BDZs and bicuculline also could not be due to the combined drug effects. Thus, as far as BDZs are concerned, the tonic phase could be a bicuculline-sensitive phenomenon involving the $GABA_A$ receptor. Augmentation of the clonic phase by a combination of BDZ and bicuculline implied that $GABA_A$ may not play the major role in mediating the clonic component of this phenomenon.

On the other hand, in combined treatment with BDZs, picrotoxin increased the latency of onset and had a tendency to antagonize the duration of the tonic component and the frequency of clonic convulsions. It did not antagonize the duration of the clonic component or the number of the clonic convulsions, but rather augmented them. Picrotoxin (3 mg/kg) alone shortened the duration of the tonic phase and increased the duration of the clonic phase of PDC significantly. Thus the apparent blockade of the tonic phase or augmentation of the clonic phase by combinations of BDZs with picrotoxin could be due to the additivity of drug effects.

TABLE 5

ANTAGONISM FOR THE EFFECT OF GABA AGONISTS ON THE PDC BY BICUCULLINE, PICROTOXIN AND Ro 15-1788

Dose (mg/kg)		Onset Sec	Tonic Sec	Clonic Sec	Number	Frequency
Control	n=33	2.4 ± 0.1	3.6 ± 0.4	13.6 ± 0.5	17.6 ± 0.6	1.31 ± 0.03
AOAA (30)	n=10	1.9 ± 0.2*	6.8 ± 0.9‡	15.3 ± 1.3	20.5 ± 1.0*	1.56 ± 0.05‡
Muscimol (2)	n=10	3.1 ± 0.4†	2.6 ± 0.6	15.3 ± 1.0	23.9 ± 1.3‡	1.59 ± 0.09
AOAA (30) + bicuculline (4)	n=10	2.2 ± 0.2	4.4 ± 0.8	13.9 ± 0.9	16.5 ± 1.3	1.20 ± 0.05#
AOAA (30) + picrotoxin (2)	n=10	2.5 ± 0.2†	4.0 ± 0.6§	16.0 ± 1.0*	19.1 ± 0.9	1.21 ± 0.04#
Bicuculline (4) + muscimol (2)	n=10	2.8 ± 0.1	0.7 ± 0.2‡**	16.5 ± 0.4†	20.4 ± 0.7†**	1.24 ± 0.03††
Ro 15-1788 (15) + muscimol (2)	n=10	2.6 ± 0.1	1.0 ± 0.5‡	16.6 ± 0.9†	21.0 ± 0.9†	1.28 ± 0.04
Ro 15-1788 (10)	n=10	2.0 ± 0.2	4.8 ± 0.7	11.2 ± 1.3*	17.3 ± 1.2	1.62 ± 0.09‡
Bicuculline (4)	n=10	2.4 ± 0.2	5.2 ± 0.6*	11.0 ± 0.7*	15.1 ± 0.9*	1.38 ± 0.07
Picrotoxin (2)	n=10	2.4 ± 0.2	4.4 ± 0.6	14.0 ± 0.6	18.0 ± 0.6	1.29 ± 0.04
Picrotoxin (3)	n=10	2.3 ± 0.1	1.9 ± 0.5*	16.6 ± 0.8†	21.0 ± 1.0*	1.27 ± 0.04

Results are shown as mean ± SEM. Tonic: the duration of the tonic component (sec); clonic: the duration of the clonic component (sec); number: the number of the clonic convulsions; frequency: the frequency of the clonic convulsions. *$p<0.05$, †$p<0.01$, ‡$p<0.001$, significant difference from control; §$p<0.05$, ¶$p<0.01$, #$p<0.001$, significant difference from AOAA; **$p<0.05$, ††$p<0.01$, significant difference from muscimol, determined by analysis of variance and subsequent t-test or Welch's method.

The differences between bicuculline and picrotoxin might be due to the fact that the inhibition of GABA by bicuculline is competitive and selective for the $GABA_A$ receptor whereas picrotoxin is noncompetitive and nonspecific [1,3].

Furthermore, AOAA which increases endogenous GABA content by inhibited GABA-transaminase [18], increased the tonic phase significantly ($p<0.001$). A GABA receptor agonist muscimol, however, did not affect the tonic phase but increased the number of volleys in the clonic phase ($p<0.001$). This discrepancy between AOAA and muscimol may be attributed to the different actions of the two compounds. One increases the level of the endogenous GABA which acts on all classes of GABA receptors, designated $GABA_A$, $GABA_B$, and $GABA_C$ [5], while the other acts exogenously and for only a class of receptors, i.e., $GABA_A$ [19].

Finally, the effect of AOAA on the tonic phase was antagonized by bicuculline ($p<0.1$) and picrotoxin ($p<0.05$). Thus, as far as the tonic phase was concerned, endogenous GABA may play a crucial role in mediating the tonic component of PDC, and the $GABA_A$ receptor may be implicated in the enhancement of the tonic component by BDZ in mice. Furthermore, BDZ (diazepam) also elevates the level of endogenous GABA in the cat spinal cord [13] and in the mouse brain [14]. Thus, the effect of BDZ on PDC and especially the tonic component may be due to GABAergic activation in the spinal cord. The reason why endogenous GABA, the inhibitory transmitter in the CNS, exerted excitatory effects on PDC is unknown but it is speculated that it might have both inhibitory and excitatory actions in the spinal cord because its direct application to spinal cord cell cultures produces depolarizing responses associated with a marked increase in membrane conductance [4].

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