EFFECTS OF BARBITURATES ON THE DECEREBRATE RIGIDITY IN RATS

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(Received March 1, 1982)

Effects of barbiturates (phenobarbital, mephobarbital, pentobarbital, hexobarbital and amobarbital) on the tonic and phasic responses of forelimbs were studied in the rats in which rigidity was induced by means of anemic decerebration. The inhibitory pattern of the tonic and phasic responses to phenobarbital was different from those to the other barbiturates, *i.e.* phenobarbital gradually inhibited both tonic and phasic responses, whereas other barbiturates suppressed significantly only the tonic response immediately after the administration of a relatively low dose (10 mg/kg) but hardly inhibited the phasic response unless at higher doses (20 and 40 mg/kg). Possible involvement of GABA-system in the inhibitory action of phenobarbital and pentobarbital on the rigidity was investigated. Since inhibition of the phasic response to pentobarbital was potentiated by the pretreatment of animals with carboxymethoxylamine hemihydrochloride (AOAA) and suppressed by the pretreatment with semicarbazide, it was suggested that GABAergic mechanism may be involved in the particular inhibition of phasic response to pentobarbital. However, the inhibitory action of phenobarbital appeared to be independent of GABA-system.

Keywords — anemic rigidity; phenobarbital; mephobarbital; pentobarbital; hexobarbital; amobarbital; GABA-system

INTRODUCTION

In the experimental rigidity due to anemic decerebration in rats, increased tension (phasic response) is superimposed on the persistent rigidity (tonic response) of the forelimbs following the mechanical stimulation given to the hindlimbs.¹⁾ We reported previously that some of the centrally acting drugs showed the characteristic pattern of inhibition of these responses.^{1,2)} The drugs tested so far were classified into three groups according to the mode of action: group 1 includes mephensin and tolperisone depressing both tonic and phasic responses, group 2 includes orphenadrine and chlorpromazine which depress the tonic response and augment the phasic response, and group 3 includes benzodiazepines which depress the phasic response selectively without affecting the tonic response.¹⁾ Thus, the animal model of anemic decerebrate rigidity will give valuable knowledge about drugs acting

on the central nervous system.

Barbiturates have pharmacological actions such as anesthetic, sedative and anticonvulsant actions. Many works indicate that GABAergic mechanism may be involved in the pharmacological action of barbiturates. Macdonald and Barker³⁾ reported that in cultured mammalian neurons pharmacological activities of anesthetic barbiturates in terms of the effect on postsynaptic action of GABA and glutamate were different from those of anticonvulsant barbiturates. Furthermore, it was reported that barbiturates enhanced GABA binding in the rat brain synaptosome^{4,5)} and also GABA-mediated actions in the synapses of mammalian central nervous system,⁶⁻⁸⁾ mammalian and amphibian sympathetic ganglion cells⁹⁻¹¹⁾ and mammalian spinal cultured cells.^{3,12,13)}

In the present work, we intended to extend our knowledge about pharmacological actions

of barbiturates, using the anemic decerebrate rigidity in rats. Correlation between the GABA-system and actions of phenobarbital and pentobarbital was also examined.

MATERIALS AND METHODS

The experiments were performed on male Wistar rats weighing 250–460 g (Nippon Rat Co.). The procedure for preparing the anemic decerebrate rigidity was the same as in the previous reports.^{1,14} Briefly, after anesthetizing rats with ether, the common carotid arteries were ligated bilaterally and the occipital bone was exposed by scraping off the adhering muscle. The basilar artery was cauterized with a bipolar pin-cette coagulator electrodes (Mizuhoika Micro 1C) after making a trephined hole at the central part of the occipital bone. When anesthesia was discontinued, rigidity developed within 30 min. Records of the tonic and phasic responses were taken for 60 min after the administration of drugs (phenobarbital, mephobarbital, pentobar-

bital, hexobarbital, and amobarbital), and the effects of drugs were obtained by comparing the mean of the tension for each 5 min before indicated time with the mean for 10 min before administration (control value).

Effects of barbiturates on the GABA-system were examined using phenobarbital and pentobarbital. Picrotoxin (2 mg/kg) was injected at 10 min after the administration of barbiturates. Carboxymethoxylamine hemihydrochloride (AOAA) (10 mg/kg) and semicarbazide hydrochloride (200 mg/kg) were administered intravenously 2 and 4 h before the administration of barbiturates. Statistical analysis of the data was carried out according to Student's *t*-test.

Drugs used were mephobarbital Na, hexobarbital and semicarbazide hydrochloride (Tokyo Kasei), phenobarbital Na (Daiichi), amobarbital Na (Serva Feinbiochemica), picrotoxin (Wako Pure Chem.) and AOAA (Aldrich Chemicals). Mephobarbital and hexobarbital were dissolved in 0.5N NaOH solution and then

FIG. 1. *Effect of Phenobarbital Na (20 mg/kg, i.v.) on Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats*

Left: A typical drawing of the effect of phenobarbital. An arrow indicates when the drug was given i.v. Abscissa: Time after administration (min). Ordinate: Tension (g).

Right: Time course of the response. Abscissa: Time after drug administration. Ordinate: Tension in percentage of the control. Each point represents the mean of 5 separate experiments, with the S.E.M. indicated. (●) tonic response, (○) phasic response.

adjusted to pH 10 with 0.2N HCl solution before the administration. Other compounds were dissolved in physiological saline. The concentration of the drug solution employed was such that the dose administered always represented 0.05 ml/100 g body weight. Drugs were administered through a cannula inserted into the femoral vein.

RESULTS

1. Effects of Barbiturates

In order to evaluate the action of barbiturates on the tonic and phasic responses in the anemic decerebrate rigidity of rats, the doses of 10, 20 and in some experiments 40 mg/kg of five kinds of barbiturates were injected intravenously.

Phenobarbital Na (10 mg/kg) inhibited grad-

FIG. 2. *Effect of Mephobarbital (20 mg/kg, i.v.) on Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats*
Legends are the same as in Fig. 1.

FIG. 3. *Effect of Pentobarbital Na (20 mg/kg, i.v.) on Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats*
Legends are the same as in Fig. 1.

usually the tonic response without affecting the phasic response ($N=5$). A higher dose of 20 mg/kg showed a marked inhibition on the tonic

response, and a smaller inhibition on the phasic response (Fig. 1).

The effect of mephobarbital (20 mg/kg) is

FIG. 4. *Effect of Hexobarbital (20 mg/kg, i.v.) on Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats*
Legends are the same as in Fig. 1.

FIG. 5. *Effect of Amobarbital Na (20 mg/kg, i.v.) on Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats*
Legends are the same as in Fig. 1.

shown in Fig. 2. Mephobarbital is known as an anticonvulsant barbiturate. Tonic response was inhibited immediately after the administration of the drug and did not recover to the control level for the observation period of 60 min, and, in contrast, phasic response was not inhibited. The same inhibitory pattern was observed even at a lower dose of 10 mg/kg of mephobarbital ($N=5$). However, by a higher dose of 40 mg/kg, both responses were inhibited significantly ($N=5$).

An effective anesthetic, pentobarbital Na (20 mg/kg), suppressed both the tonic and phasic responses immediately after the administration (Fig. 3). However, 10 mg/kg of pentobarbital

FIG. 6. Effect of Picrotoxin (2 mg/kg, i.v.) on Phenobarbital Na (20 mg/kg, i.v.)-induced Suppression of the Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats

(●) tonic response of phenobarbital-administered group, (○) phasic response of phenobarbital-administered group, (▲) tonic response of picrotoxin and phenobarbital-administered group, and (△) phasic response of picrotoxin and phenobarbital-administered group. Each point represents the mean of 5 separate experiments, with the S.E.M. indicated. Abscissa: Time after phenobarbital administration (min). An arrow indicates the point when picrotoxin was given i.v. Ordinate: Tension (%) when expressed the average of tension developed for 10 min before drug administration as 100%.

Na inhibited only the tonic response ($N=5$).

An ultrashort-acting anesthetic, hexobarbital (20 mg/kg), inhibited phasic response for a very short period and the recovery of the phasic response was rapid (Fig. 4). The inhibition of the tonic response was rather prolonged. With a lower dose of 10 mg/kg, the phasic response remained unaffected whereas tonic response was inhibited significantly ($N=5$).

The effect of amobarbital Na (20 mg/kg) is shown in Fig. 5. An intermediate-acting anesthetic, amobarbital Na, 10 and 20 mg/kg, inhibited only the tonic response. However, with a higher dose of 40 mg/kg, the suppression of phasic response was observed ($N=5$).

These results mentioned above indicate that the inhibition pattern produced by barbiturates is dose-dependent except for phenobarbital. The gradual increase in the degree of inhibition of the responses to phenobarbital was notable, be-

FIG. 7. Effect of Picrotoxin (2 mg/kg, i.v.) on Pentobarbital Na (20 mg/kg, i.v.)-induced Suppression of the Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats

(●) tonic response of pentobarbital-administered group, (○) phasic response of pentobarbital-administered group, (▲) tonic response of picrotoxin and pentobarbital-administered group, and (△) phasic response of picrotoxin and pentobarbital-administered group. Abscissa: Time after pentobarbital administration. Others are the same as in Fig. 6.

cause the other barbiturates produced a rapid inhibition following the administration. At 10 mg/kg, all barbiturates except phenobarbital suppressed the tonic response rather selectively and, when the dose was increased, the effect was extended to suppress the phasic response. A dose of 20 mg/kg of pentobarbital Na significantly inhibited the phasic response, whereas the same dose of other barbiturates except phenobarbital hardly suppressed the phasic response (suppression by hexobarbital was very small). These facts suggest that the pharmacological action of pentobarbital is characteristic among barbiturates tested. However, the suppression of phasic response to mephobarbital and amobarbital was

detected when a higher dose (40 mg/kg) of these barbiturates were administered, and the inhibitory patterns of phasic and tonic responses to amobarbital and mephobarbital were almost similar to that of pentobarbital. Therefore, it may be considered that pentobarbital has a potent inhibitory action on the phasic response compared with other barbiturates tested.

2. Correlation with GABA-system

Many works indicate that barbiturates have an action on the GABA-system as mentioned in the introduction. Thus, it was examined in the following experiments to see whether barbiturates exerted the suppression of tonic and/or phasic responses through acting on GABA-system in the anemic decerebrate rats.

1) Effect of Picrotoxin—When picrotoxin (2

FIG. 8. Effect of AOAA Treatment (10 mg/kg, i.v.) on Phenobarbital Na (10 mg/kg, i.v.)-induced Suppression of the Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats (●) tonic response of phenobarbital-administered group, (○) phasic response of phenobarbital-administered group, (▲) tonic response of AOAA and phenobarbital-administered group, and (△) phasic response of AOAA and phenobarbital-administered group. Each point represents the mean of 4–5 separate experiments, with the S.E.M. indicated. Abscissa: Time after phenobarbital administration (min). Ordinate: Tension (%) when expressed the average of tension developed for 10 min before drug administration as 100%.

FIG. 9. Effect of AOAA Treatment (10 mg/kg, i.v.) on Pentobarbital Na (10 mg/kg, i.v.)-induced Suppression of the Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats (●) tonic response of pentobarbital-administered group, (○) phasic response of pentobarbital-administered group, (▲) tonic response of AOAA and pentobarbital-administered group, and (△) phasic response of AOAA and pentobarbital-administered group. Abscissa: Time after pentobarbital administration (min). Others are the same as in Fig. 8. Statistical difference (a) $p < 0.05$, (b) $p < 0.01$, calculated according to Student's *t*-test).

mg/kg) was administered 10 min after phenobarbital Na (20 mg/kg) treatment, alterations in both the tonic and phasic responses were not affected, suggesting that the inhibition of tonic and phasic responses to phenobarbital is not brought about through GABA-system (Fig. 6). Picrotoxin (2 mg/kg) did not affect the recovery pattern of pentobarbital-suppressed tonic response. However, the phasic response suppressed by pentobarbital Na (20 mg/kg) appeared to recover rather earlier in the picrotoxin-administered group (Fig. 7).

2) *Effect of AOAA*—The intravenous or intraperitoneal administration of AOAA produces a marked increase of GABA content in the cen-

tral nervous system.^{15,16} In the present study, both tonic and phasic responses were developed in the rats pretreated with AOAA (10 mg/kg, 2 h before barbiturates administration). The inhibition of tonic and phasic responses induced by phenobarbital was not affected by pretreatment of animals with AOAA (Fig. 8). However, although not affected significantly on the tonic response, pretreatment with AOAA did induce a remarkable enhancement of the inhibition of phasic response due to pentobarbital (Fig. 9).

3) *Effect of Semicarbazide*—The treatment with semicarbazide has been demonstrated to produce a marked decrease in GABA content *via* inhibition of glutamic acid decarboxylase activity in the central nervous system.^{17,18} In the present study, the tonic and phasic responses were developed after the treatment of animals with semicarbazide hydrochloride (200 mg/kg,

FIG. 10. *Effect of Semicarbazide Hydrochloride Treatment (200 mg/kg, i.v.) on Phenobarbital Na (20 mg/kg, i.v.)-induced Suppression of the Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats*

(●) tonic response of phenobarbital-administered group, (○) phasic response of phenobarbital-administered group, (▲) tonic response of semicarbazide and phenobarbital-administered group, and (△) phasic response of semicarbazide and phenobarbital-administered group. Each point represents the mean of 5 separate experiments, with the S.E.M. indicated. Abscissa: Time after phenobarbital administration (min). Ordinate: Tension (%) when expressed the average of tension developed for 10 min before drug administration as 100%.

FIG. 11. *Effect of Semicarbazide Hydrochloride Treatment (200 mg/kg, i.v.) on Pentobarbital Na (20 mg/kg, i.v.)-induced Suppression of the Tonic and Phasic Responses of Forelimbs in Anemic Decerebrate Rats*

(●) tonic response of pentobarbital-administered group, (○) phasic response of pentobarbital-administered group, (▲) tonic response of semicarbazide and pentobarbital-administered group, and (△) phasic response of semicarbazide and pentobarbital-administered group. Abscissa: Time after pentobarbital administration (min). Others are the same as in Fig. 10. Statistical difference (a) $p < 0.05$, calculated according to Student's *t*-test).

4 h before barbiturates administration). There was no effect of semicarbazide pretreatment on the suppression of either response attributed to a dose of 20 mg/kg of phenobarbital Na (Fig. 10). On the other hand, pretreatment with semicarbazide produced a slight reduction in the degree of inhibition of the phasic response by pentobarbital Na (20 mg/kg) without affecting the changes in the tonic response (Fig. 11).

DISCUSSION

The present study indicates that phenobarbital shows an inhibitory action with a different pattern from the other barbiturates on the tonic and phasic rigidity responses in anemic decerebrate rats. Inhibitory pattern produced by pentobarbital was characteristic at the dose of 20 mg/kg, that is, the inhibition of both responses was rapid. Macdonald and Barker³⁾ demonstrated in their electrophysiological studies on mouse cultured spinal cells that anesthetic barbiturates such as pentobarbital and secobarbital had more potent inhibitory effect on the spontaneous firing and the membrane conductance than anticonvulsant barbiturates such as phenobarbital and mephobarbital. In the present study, however, we could not find the corresponding difference in the pattern of inhibitory effects on decerebrate-induced rigidity between the clinically classified two groups, since the inhibitory pattern produced by mephobarbital which has been used as an anticonvulsant was similar to that of anesthetic barbiturates such as amobarbital.

It has been reported that barbiturates enhance GABA action in the various experimental systems.³⁻¹³⁾ In the present work, barbiturates suppressed the tonic and phasic responses of the anemic rigidity.

Phenobarbital produced a characteristic pattern on this model. In the presence of picrotoxin, a GABA antagonist, only the inhibition of phasic response to pentobarbital was inclined to be reversed. AOAA is known to increase GABA content in the central nervous systems by inhibiting GABA-transaminase.^{15,16)} The pretreat-

ment with AOAA augmented the suppression of phasic response induced by pentobarbital, whereas there was no augmentation by AOAA of the inhibition of phasic response produced by phenobarbital. The pretreatment with semicarbazide, an inhibitor of glutamic acid decarboxylase, reduced only the inhibition of phasic response to pentobarbital for a very short period. These results indicate that GABA system may be involved in the inhibitory action of pentobarbital on the phasic response. It may be suggested, on the other hand, that GABA-system is not responsible for the inhibition of the tonic response to pentobarbital, since the treatment with AOAA or semicarbazide showed no effect of pentobarbital-induced suppression of the tonic response.

Macdonald and Barker³⁾ reported that augmentation of GABA action by pentobarbital was stronger than that of phenobarbital. Ticku and Olsen¹⁹⁾ reported that the ability of pentobarbital in displacing [³H]-dihydropicrotoxin, the binding site of which was considered to be coupled with GABA receptor, was stronger than that of phenobarbital. In the present work, the suppression of the tonic and phasic responses to phenobarbital was not affected by the treatment with AOAA or semicarbazide. Thus, the involvement of GABA-system in the inhibitory action of phenobarbital on the phasic response may be scarce or, if present, smaller in extent than in the case of pentobarbital.

It was reported that some areas including the medulla and the spinal cord were supplied with blood flow in the anemic decerebrate rats, whereas other parts of central nervous systems above the medulla sustained the anemic state.²⁰⁾ The rigidity in this animal model is due to hyperactivity of α -motoneurons. It was also reported that the development of the phasic response of the forelimb rigidity was associated with a mechanical stimulation on cutaneous afferents and spinalization diminished the phasic responses.¹⁾ The induction of the phasic response can be attributed to hyperactivity of a system involving the cutaneous afferents. Our previous studies

suggested that the tonic responses of the rigidity might be related to the descending pathway from the reticular formation of the medulla, and the phasic responses might be associated with the descending pathway facilitated by cutaneous afferents.¹⁾

The cuneate nucleus is a region of central nervous systems receiving the peripheral afferent input where pre- and post-synaptic GABA systems are involved as an synaptic inhibition.^{21,22)} It was reported that phenobarbital slightly enhanced pre- and post-synaptic inhibition and reduced the excitatory processes in the cuneatus nucleus of cats.²³⁾ Barbiturates have been considered to affect the activity of the reticular formation of the medulla which exerts an activating and an inhibitory influence on the activity of various parts of central nervous systems.²⁴⁾ Although a precise mechanism of barbiturates on the rigidity remains obscure, a possible explanation may be that the suppression of the phasic response to pentobarbital is exerted through the action on the cuneate nucleus where pentobarbital enhances the inhibitory GABA-system. The suppression of the tonic response to barbiturates may be ascribed to the suppression of the activity of the brain stem reticular formation. However, a mechanism to explain the facts that phenobarbital slightly inhibited the phasic response without being influenced by AOAA and semicarbazide, remained to be elucidated further.

Acknowledgements This work was supported in part by a Scientific Research Grant from the Ministry of Education, Science and Culture. The technical advice of Dr. Togari, Department of Life Chemistry, Tokyo Institute of Technology, is gratefully acknowledged.

REFERENCES

- 1) A.Togari, Y.Kudo and H.Fukuda: Effects of centrally acting muscle relaxants on the anemic decerebrate rigidity in rats, *J. Pharm. Dyn.*, **1**, 332–337 (1978).
- 2) H.Fukuda, Y.Kudo and A.Togari: Effects of diazepam and baclofen on the anemic decerebrate rigidity in rats, *Folia Pharmacol. Jpn.*, **75**, 535–542 (1979).
- 3) R.L.Macdonald and J.L.Barker: Anticonvulsant and anesthetic barbiturates: different postsynaptic actions in cultured mammalian neurones, *Neurology*, **29**, 432–447 (1979).
- 4) M.Willow and G.A.R.Johnston: Enhancement of GABA binding by pentobarbitone, *Neurosci. Lett.*, **18**, 323–327 (1980).
- 5) M.Willow and G.A.R.Johnstone: Enhancement by anesthetic and convulsant barbiturates of GABA binding to rat brain synaptosomal membranes, *J. Neurosci.*, **4**, 364–367 (1981).
- 6) D.R.Curtis and D.Lodge: Pentobarbitone enhancement of the inhibitory action of GABA, *Nature (London)*, **270**, 543–544 (1977).
- 7) D.Lodge and D.R.Curtis: Time course of GABA and glycine actions on cat spinal neurones: effect of pentobarbitone, *Neurosci. Lett.*, **8**, 125–129 (1978).
- 8) M.L.Mayer: A barbiturate evoked intensification of post-synaptic inhibition in the preoptic ovulatory trigger region of the hypothalamus, *Brain Res.*, **207**, 229–233 (1981).
- 9) D.A.Brown and A.Constanti: Interaction of pentobarbitone and γ -aminobutyric acid on mammalian sympathetic ganglion cells, *Br. J. Pharmacol.*, **63**, 217–224 (1978).
- 10) R.A.Nicoll: Pentobarbital: differential postsynaptic actions on sympathetic ganglion cells, *Science*, **199**, 451–452 (1978).
- 11) R.H.Evans: Potentiation of the effects of GABA by pentobarbitone, *Brain Res.*, **171**, 113–120 (1979).
- 12) R.L.Macdonald and J.L.Barker: Different action of anti-convulsant and anesthetic barbiturates revealed by use of cultured mammalian neurones, *Science*, **200**, 775–777 (1979).
- 13) D.W.Schulz and R.L.Macdonald: Barbiturate enhancement of GABA-mediated inhibition and activation of chloride ion conductance: correlation with anticonvulsant and anesthetic actions, *Brain Res.*, **209**, 177–188 (1981).
- 14) H.Fukuda, Y.Kudo and A.Togari: Methods for measurement of experimental rigidity, *Pharmacometrics*, **18**, 1063–1066 (1979).
- 15) J.A.Bell and E.G.Anderson: Dissociation between aminooxyacetic acid-induced depression of spinal reflexes and the rise in cord GABA levels, *Neuropharmacology*, **13**, 885–894 (1974).
- 16) E.Carmona, C.Gomes and G.Trolin: Effect of aminooxyacetic acid (AOAA) on GABA levels in some parts of rat brain, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **312**, 51–55 (1980).
- 17) J.D.Wood and D.E. Abraham: The comparative effect of various hydrazides on γ -aminobutyric acid and its metabolism, *J. Neurochem.*, **18**, 1017–1025 (1971).
- 18) J.A.Bell and E.G.Anderson: The influence of semicarbazide-induced depletion of γ -aminobutyric acid on presynaptic inhibition, *Brain Res.*, **43**,

- 161–169 (1972).
- 19) M.K.Ticku and R.W.Olsen: Interaction of barbiturates with dihydropicrotoxinin binding site related to the GABA receptor-ionophore system, *Life Sci.*, **22**, 1643–1653 (1978).
- 20) H.Fukuda, T.Ito, S.Hashimoto and Y.Kudo: Rigidity in rats to anemic decerebration and the effect of chlorpromazine, *Japan. J. Pharmacol.*, **24**, 810–813 (1974).
- 21) J.S.Kelly and L.P.Renaud: On the pharmacology of ascending, descending and recurrent postsynaptic inhibition of the cuneo-thalamic relay cells in the cat, *Br. J. Pharmacol.*, **48**, 396–408 (1973).
- 22) D.R.Curtis and G.A.R.Johnston: Amino acid transmitters in the mammalian central nervous system, *Rev. Physiol., Biochem. Exp. Pharmacol.*, **69**, 97–188 (1974).
- 23) P.Polc and W.Haefely: Effects of two benzodiazepines, phenobarbitone, and baclofen on synaptic transmission in the cat cuneate nucleus, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **294**, 121–131 (1976).
- 24) V.V.Zakusov: "Pharmacology of central synapses," Pergamon Press, Oxford, 1980, p. 26.