Pharmacology Biochemistry & Behavior, Vol. 32, pp. 357–360. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

BRIEF COMMUNICATION

Effects of GABA Antagonists on the Pentobarbital-Induced Depression of Respiration and Cough in Rats

JUNZO KAMEI, MASARU OGAWA AND YUTAKA KASUYA

Department of Pharmacology, School of Pharmacy, Hoshi University
2-4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan

Received 19 January 1988

KAMEI, J., M. OGAWA AND Y. KASUYA. *Effects of GABA antagonists on the pentobarbital-induced depression of respiration and cough in rats.* PHARMACOL BIOCHEM BEHAV 32(1) 357–360, 1989.—In order to determine the possible involvement of GABA-ergic mechanisms in the modulation of the cough reflex, the effects of GABA antagonists on the pentobarbital-induced depression of respiration and cough were examined in a comparative study in rats. The cough reflex was induced by application of electrical stimulation to the tracheal mucosa by the puncture electrode-induced cough method. The 50% antitussive dose (AtD50) of pentobarbital was calculated by the "up and down" method. Pentobarbital (10 mg/kg, IP) caused a reduction of tidal volume, which was counteracted by pretreatment with picrotoxin (3 mg/kg, IP) or bicuculline (3 mg/kg, IP). However, neither picrotoxin nor bicuculline were able to counteract the reduction in frequency of respiration. The AtD50 of pentobarbital was 1.95 mg/kg when administered IP. The AtD50 of pentobarbital was not altered after pretreatment of rats with picrotoxin (1.85 mg/kg, IP) or with bicuculline (1.55 mg/kg). These results suggest that GABA-ergic mechanisms may not be involved in the cough-depressant effect of pentobarbital.

Respiration Cough reflex GABA Pentobarbital Picrotoxin Bicuculline

OUR previous studies have shown that the monoamine neurotransmitters appear to be involved in the central generating mechanisms of the cough reflex (7–10). Recently, it has also been proposed that certain amino acids, in particular gamma-aminobutyric acid (GABA), may be involved in regulation of respiration (11–13, 19). However, the involvement of GABA-ergic systems in the central generating mechanisms of the cough reflex is still unknown.

Pentobarbital has been shown to enhanced GABA-ergic neurotransmission by increasing conductance at the Cl^- ionophore. Several reports have indicated that pentobarbital causes depression of respiration by interacting with GABA-ergic mechanisms (3, 6, 20). Pentobarbital has also been demonstrated to depress the cough reflex (14), but the mechanism of this depression is not fully understood.

The present study was undertaken to ascertain whether the cough-depressant effect of pentobarbital involves modulation of GABA-ergic neurotransmission. We examined the ability of picrotoxin and bicuculline, a putative blocker of GABA receptors, to counteract the depression of respiration and the cough reflex by pentobarbital.

METHOD

Male Sprague-Dawley rats (65 animals) weighing 250–360 g were used in the experiments. The animals were housed under regulated dark-light condition and fed on a standard laboratory rodent diet and tap water ad lib.

Measurement of Respiration

The rats were anesthetized with α-chloralose (70 mg/kg, IP). An incision was made through the strap muscles of the neck to expose the trachea, and a tracheal cannula was inserted into the caudal site of the transected trachea. The animals were able to breath spontaneously through this cannula. Respiration was recorded from the tracheal cannula by a pneumotachograph (Nihon Kohden, TP-602T) connected to polygraph (Nihon Kohden, RM-6100), which permitted quantification of the frequency of respiration (RF). Tidal volume (Vt) was quantified by integration of the signal which is obtained from the pneumatochograph. For rats in the range of 250–360 g, Vt varies approximately 35% between animals. To minimize this variation we have correlated Vt with body weight. As a standard procedure we have, therefore, chosen to describe VT as Vt/kg (ml/kg). Measurement of respiration was started after a minimum stabilization period of 15 min. Ten mg/kg of pentobarbital was given IP as a bolus injection. Control animals were given corresponding volumes of saline (0.1 ml/100 g body weight).

Induction of the Cough Reflex

The cough reflex was induced by electrical stimulation, by the puncture electrode-induced cough method as described previously (10). The puncture electrode was made of stainless steel wire (0.2 mm diameter, 10 cm length) and coated with epoxy for insulation. Before use, the tip of the puncture electrode was exposed. The electrode was inserted into the trachea through a guiding cannula (injection needle 23-ga, Terumo). The guiding cannula was pulled out as soon as the electrode had been inserted into the trachea. A stainless steel needle, placed arbitrarily into the muscle behind the ear, was used as the indifferent electrode. The electrical stimulation used for inducing the cough reflex consisted of a square-wave pulse with a frequency of 40 Hz; the duration of the pulse was 1 msec; the voltage was 2–4 V; and the duration of application was 10 sec. The stimulus intensity for each animal was set by increasing the voltage. The tip of the electrode was placed near the bifurcatio tracheae, because the bifurcatio tracheae is the most sensitive site for stimulation of coughing. The thoracic movements of the rat were measured by a force-displacement transducer (Nihon Kohden, 611T), and used as an indicator of the cough reflex. Recordings were made on a polygraph (Nihon Kohden, RM-6100).

Calculation of the 50% Antitussive Dose (AtD50) of Pentobarbital

The AtD50 was calculated by the "up and down" method (4) according to the procedure as described previously (10). The electrical stimuli used for inducing the cough reflex were given at intervals of 5, 10, 15, 30, 45, and 60 min after administration of pentobarbital. When no cough reflex occurred in response to even one stimulus, the pentobarbital was regarded as effective. When the cough reflex occurred in response to all stimuli, the pentobarbital was regarded as ineffective. Ten animals were used in each experiment to calculate the AtD50. Only 1 dose of pentobarbital was given to each animal. Each animal was tested at a dose immediately below and/or a dose immediately above the dose used in the previous test, with the results of the previous test judged as outlined above. The ratio between the dose of pentobarbital tested in this experiment was 1.3.

Drugs

The following drugs were used in this study: pentobarbital sodium (Tokyo Kasei, Tokyo, Japan), picrotoxin (Sigma Chemical Co., St. Louis, MO), bicuculline methiodide (Sigma Chemical Co., St. Louis, MO) and strychnine hydrochloride (Sigma Chemical Co., St. Louis, MO). All drugs were dissolved in saline immediately before use. Doses of all drugs are expressed in terms of the base. Picrotoxin (3 mg/kg) and strychnine (1 mg/kg) were administered 5 min before pentobarbital. Bicuculline (3 mg/kg) was administered simultaneously with pentobarbital. The doses of picrotoxin, strychnine and bicuculline used in this experiment were determined with reference to the previous reports (1, 15, 17).

Statistical Analysis

Statistical analysis of the results was performed by analysis of variance (ANOVA). p Values of less than 0.05 were considered significant.

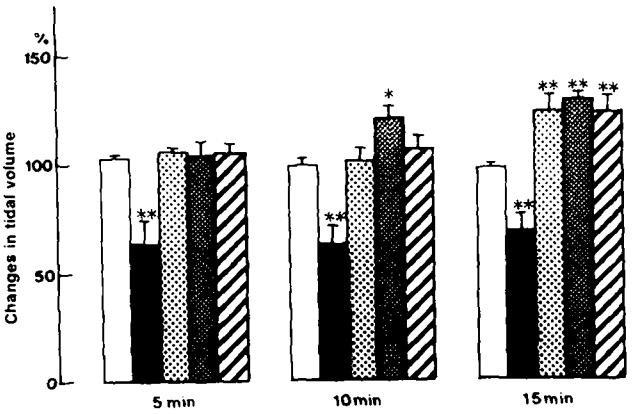

FIG. 1. Effects of 3 mg/kg picrotoxin (lightly-shaded bars), 3 mg/kg bicuculline (heavily-shaded bars), and 1 mg/kg strychnine (hatched bars) on 10 mg/kg pentobarbital- (solid bars) induced decrease in tidal volume. Each column represents the mean with S.E. of results from five experiments. All drug were injected intraperitoneally. An asterisk indicates that the changes are significant at $p<0.05$; two asterisks indicate that the changes are significant at $p<0.01$ when compared with the effect of saline (open bars).

RESULTS

Effects of Pentobarbital on Resting Respiration

No changes in tidal volume (Vt) or frequency of respiration (RF) were found following administration of saline. Administration of pentobarbital (10 mg/kg) induced a decrease in Vt and RF. A significant decrease in both Vt and RF was seen 5, 10, and 15 min after administration of pentobarbital (Figs. 1 and 2). After 60 min, the RF remained significantly lower in treated animals than in the control animals which received saline, while the Vt returned to control levels within this time.

Effects of Picrotoxin on the Pentobarbital-Induced Depression of Respiration

Picrotoxin (3 mg/kg) by itself had no significant effect on either Vt (without picrotoxin: 3.7±0.3 ml/kg; treatment with picrotoxin: 3.6±0.1 ml/kg, n=5) or RF (without picrotoxin: 83.6±3.5 breath/min; treatment with picrotoxin: 86.8±5.2 breath/min, n=5) within 5 min after administration. As shown in Fig. 1, pretreatment of picrotoxin counteracted the reduction of Vt produced by pentobarbital. By contrast, Vt was significantly increased in picrotoxin-treated rats compared with the Vt in saline-treated control, 15 min after administration of pentobarbital. It is, however, noteworthy that although 3 mg/kg of picrotoxin effectively restored the Vt, the pentobarbital-induced depression of RF was not completely reversed (Fig. 2).

Effects of Bicuculline on the Pentobarbital-Induced Depression of Respiration

Bicuculline (3 mg/kg) by itself had no significant effect on either Vt (without bicuculline: 3.7±0.5 ml/kg; treatment with bicuculline: 3.9±0.4 ml/kg, n=5) or RF (without bicuculline:

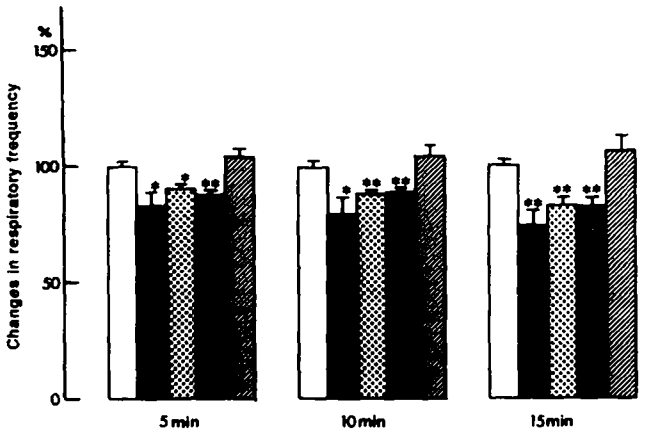

FIG. 2. Effects of 3 mg/kg picrotoxin (lightly-shaded bars), 3 mg/kg bicuculline (heavily-shaded bars), and 1 mg/kg strychnine (hatched bars) on 10 mg/kg pentobarbital- (solid bars) induced decrease in frequency of respiration. Each column represents the mean with S.E. of results from five experiments. All drugs were injected intraperitoneally. An asterisk indicates that the changes are significant at $p<0.05$; two asterisks indicate that the changes are significant at $p<0.01$ when compared with the effect of saline (open bars).

86.3±2.9 breath/min; with bicuculline: 87.0±3.2 breath/min, n=5) within 1 min. In the presence of bicuculline, the pentobarbital-induced depression of Vt was markedly reduced (Fig. 1). In fact, Vt was significantly increased in bicuculline-treated rats compared with the Vt in saline-treated control, 10 and 15 min after administration of pentobarbital (Fig. 1). However, bicuculline failed to alter the reduction of RF produced by pentobarbital (Fig. 2). The RF was significantly reduced by pentobarbital after pretreatment with bicuculline.

Effects of Strychnine on the Pentobarbital-Induced Depression of Respiration

Vt (without strychnine: 3.5±0.6 ml/kg; treatment with strychnine: 4.8±1.0 ml/kg, n=5) and RF (without strychnine: 85.8±4.6 breath/min; treatment with strychnine: 95.0±6.5 breath/min, n=5) were both increased 5 min after administration of strychnine. However, these changes were not significant. Strychnine counteracted the effects of pentobarbital on the Vt and RF (Figs. 1 and 2). Furthermore, Vt in strychnine-treated rats was significantly greater than in saline-treated controls, 15 min after administration of pentobarbital (Fig. 1). The effect was of short duration, and 30 min after the administration of strychnine, values of Vt and RF were not significantly larger than in the animals receiving pentobarbital alone.

Effects of Picrotoxin, Bicuculline, and Strychnine on the Cough-Depressant Effect of Pentobarbital

The electrical stimuli applied to the tracheal mucosa, generated by the puncture electrode, produced coughs (2–3 coughs/stimulation) stably and reproducibly.

As shown in Table 1, the AtD50 of pentobarbital was 1.95 mg/kg. The range of doses of pentobarbital in strychnine-

TABLE 1

EFFECT OF PRETREATMENT WITH PICROTOXIN, BICUCULLINE, AND STRYCHNINE ON THE ANTITUSSIVE EFFECT OF PENTOBARBITAL

Pretreatment, IP	AtD50 of Pentobarbital (mg/kg, IP)
Saline	1.95 (1.44–2.65)*
Picrotoxin 3 mg/kg	1.84 (1.16–2.93)
Bicuculline 3 mg/kg	1.55 (1.05–2.28)
Strychnine 1 mg/kg	3.91 (3.13–4.90)

AtD50: 50% antitussive dose. AtD50 values were determined by the "up and down" method. Ten animals were used at each experiment to calculate the AtD50.

*Numbers in parentheses show 95% confidence limits of AtD50.

treated rats was 2.3–5.0 mg/kg. The AtD50 of pentobarbital in strychnine-treated rats (3.91 mg/kg) was 2-fold higher than the AtD50 for pentobarbital in saline-treated rats. However, the AtD50 of pentobarbital was hardly affected by pretreatment with picrotoxin (1.84 mg/kg) or with bicuculline (1.55 mg/kg). The range of doses of pentobarbital on picrotoxin- and bicuculline-treated rats was 1.3–3.0 mg/kg.

DISCUSSION

Hurle *et al.* (6) concluded that the differential effects induced by depressant drugs, such as pentobarbital, upon RF and Vt resulted from a selective interaction with structures specifically related to a particular function in the generation of the respiratory output. Our results show that pentobarbital is more effective in depressing Vt than in depressing RF. In agreement with our findings, there are some observations (5, 17, 18) that most of the pentobarbital-induced depression of respiration can be accounted for by a reduction in Vt. The finding that barbiturates interact specifically with certain sites in the GABA receptor complex (16) may explain some of the effects of these drugs. However, it is noteworthy that Yamada *et al.* (19) reported that the intracisternal administration of GABA and bicuculline had no significant effect in the respiratory timing mechanism, as indicated by the absence of any effect on the rate of respiration, the duration of inspiration, the duration of expiration, and the duration of the whole cycle. Thus, it appears that pentobarbital may not produce depression of RF by interacting with the GABA-ergic systems. The results of the present study, showing that the GABA antagonists picrotoxin and bicuculline counteract the pentobarbital-induced depression of Vt, whereas the reduction of RF is not antagonized by picrotoxin and bicuculline, support this interpretation. In agreement with our findings, Bolme and Fuxe (1) observed that picrotoxin did not antagonize the RF-depressant effect of diazepam, a drug known to increase the affinity of GABA to the receptors.

To examine a possible interaction of the GABA-ergic system with the pentobarbital-induced cough-depressant effect, picrotoxin and bicuculline were studied for their ability to antagonize the pentobarbital-induced depression of the cough reflex. The present results show that pentobarbital has a potent antitussive effect in rats. The AtD50 of pentobarbi-

tal is similar as that of dihydrocodeine (10). Picrotoxin and bicuculline were unsuccessful in reversing the pentobarbital-induced depression of the cough reflex. On the other hand, strychnine fully reversed the effects of pentobarbital on the cough reflex. Strychnine also counteracts the pentobarbital-induced depression of both Vt and RF. However, strychnine blocked the effects of glycine without affecting those of GABA (2). Furthermore, Wang *et al.* studied the effect of strychnine on the firing pattern of respiratory neurons (18). They suggested a limited role of glycine in the respiratory network. Thus, it is possible that the antagonistic effects of strychnine on the pentobarbital-induced depression of Vt, RF and cough reflex may be the consequence of a nonspecific excitatory action.

In conclusion, it seems likely that pentobarbital may not produce depression of the cough reflex by interacting with the GABA-ergic system. Moreover, the effect of pentobarbital on metabolism also needs to be addressed. It is logical to believe that the level of CO_2 in blood or tissue might be an important controller of respiration; as it decreases, ventilation might decrease. From the present study alone, however, we cannot deduce whether the pentobarbital-induced depression of the cough reflex is due to a primary effect on respiration or secondary to a decrease in metabolic function. Further studies are necessary to resolve this problem.

ACKNOWLEDGEMENTS

We are grateful to Satoshi Kimura and Yuka Dobashi for their excellent assistance.

REFERENCES

1. Bolme, P.; Fuxe, K. Possible involvement of GABA mechanisms in central cardiovascular and respiratory control. Studies on the interaction between diazepam, picrotoxin and clonidine. Med. Biol. 55:301–309; 1977.
2. Curtis, D. R.; Duggan, A. W.; Johnston, G. A. R. The specificity of strychnine as a glycine antagonist in the mammalian spinal cord. Exp. Brain Res. 12:547–565; 1971.
3. Denavit-Saubié, M.; Foutz, A. S.; Morin-Surun, M.-P.; Boundinot, E.; Champagnat, J.; Gonsalves, F. Effects of pentobarbital and flurazepam on respiratory neurons in undrugged cats. Pharmacol. Biochem. Behav. 26:647–651; 1987.
4. Dixon, W. J.; Mood, A. M. A method for obtaining and analysing sensitivity data. J. Am. Stat. Assoc. 43:109–126; 1948.
5. Hurle, M. A.; Mediavilla, A.; Florez, J. Morphine, pentobarbital and naloxone in the ventral medullary chemosensitive areas: differential respiratory and cardiovascular effects. J. Pharmacol. Exp. Ther. 220:642–647; 1982.
6. Hurle, M. A.; Dierssen, M. M.; Florez, J. Mechanism of the respiratory action of pentobarbital at the medullary and pontine levels. Eur. J. Pharmacol. 125:225–232; 1986.
7. Kamei, J.; Hosokawa, T.; Yanaura, S.; Hukuhara, T., Jr. Effects of methysergide on the cough reflex. Jpn. J. Pharmacol. 42:450–452; 1986.
8. Kamei, J.; Hosokawa, T.; Yanaura, S.; Hukuhara, T., Jr. Involvement of central serotonergic mechanisms in the cough reflex. Jpn. J. Pharmacol. 42:531–538; 1986.
9. Kamei, J.; Hukuhara, T., Jr.; Kasuya, Y. Dopaminergic control of the cough reflex as demonstrated by the effects of apomorphine. Eur. J. Pharmacol. 141:511–513; 1987.
10. Kamei, J.; Ogawa, M.; Kasuya, Y. Monoamines and the mechanisms of action of antitussive drugs in rats. Arch. Int. Pharmacodyn. 290:117–127; 1987.
11. Kneussl, M. P.; Pappagianopoulos, P.; Hoop, B.; Kazemi, H. Reversible depression of ventilation and cardiovascular function by ventriculocisternal perfusion with gamma-aminobutyric acid in dogs. Am. Rev. Respir. Dis. 133:1024–1028; 1986.
12. Kneussl, M. P.; Pappagianopoulos, P.; Hoop, B.; Kazemi, H. Effect of centrally administered γ-aminobutyric acid on metabolic function. J. Appl. Physiol. 61:472–476; 1986.
13. Lalley, P. Biphasic effects of baclofen on phrenic motoneurons: possible involvement of two types of γ-aminobutyric acid (GABA) receptors. J. Pharmacol. Exp. Ther. 226:616–625; 1983.
14. May, A. J.; Widdicombe, J. G. Depression of the cough reflex by pentobarbitone and some opium derivatives. Br. J. Pharmacol. 9:335–340; 1954.
15. Nagatani, T.; Yamamoto, T.; Sugihara, T.; Ueki, S. The effect of agonists at the GABA-benzodiazepine receptor complex on the duration of immobility of mice in the forced swimming test. Eur. J. Pharmacol. 142:17–22; 1987.
16. Olsen, R. W. Drug interactions at the GABA receptor-ionophore complex. Annu. Rev. Pharmacol. Toxicol. 22:245–277; 1982.
17. Sugimoto, T.; Takemura, M.; Sakai, A.; Ishimaru, M. Rapid transneuronal destruction following peripheral nerve transection in the medullary dorsal horn is enhanced by strychnine, picrotoxin and bicuculline. Pain 30:385–393; 1987.
18. Wang, L.; Boyarsky, L. L.; Frazier, D. T. The effect of transmitter antagonists on phasic respiratory neurons. J. Neurosci. Res. 8:657–664; 1982.
19. Yamada, K. A.; Homosh, P.; Gillis, R. A. Respiratory depression produced by activation of GABA receptors in hindbrain of cat. J. Appl. Physiol. 51:1278–1286; 1981.
20. Yamada, K. A.; Moerschbaecher, J. M.; Hamosh, P.; Gillis, R. A. Pentobarbital causes cardiorespiratory depression by interacting with GABAergic system at the ventral surface of the medulla. J. Pharmacol. Exp. Ther. 226:349–355; 1983.