Pharmacology Biochemistry & Behavior, Vol. 32, pp. 897–900. © Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Barbiturate-Induced Analgesia: Permissive Role of a $GABA_A$ Agonist

MARGARET M. McCARTHY, CARLOS BEYER AND BARRY R. KOMISARUK*

**Institute of Animal Behavior, Rutgers, The State University of New Jersey*
101 Warren Street, Newark, NJ 07102

Received 11 April 1988

McCARTHY, M. M., C. BEYER AND B. R. KOMISARUK. *Barbiturate-induced analgesia: Permissive role of a $GABA_A$ agonist.* PHARMACOL BIOCHEM BEHAV **32**(4) 897–900, 1989.—Three doses (0.025, 0.25 and 2.5 mg) of the short-acting barbiturate, pentothal, were injected intrathecally at the lumbar level of the spinal cord of female rats and did not produce analgesia in either the tail-flick latency to radiant heat (TFL) or vocalization-threshold-to-tail-shock (VTTS) tests. However, when the high dose of pentothal (2.5 mg) was given in combination with a nonanalgesia producing dose of the $GABA_A$ agonist muscimol (1 μg), a significant and prolonged analgesia was produced in both the VTTS and TFL tests, lasting up to one hour postinjection. Intrathecal injection of the intermediate dose of pentothal (0.25 mg) in combination with 1 μg muscimol also produced significant analgesia in the TFL but not the VTTS test. We suggest that barbiturates may act on spinal nociceptive pathways to reduce pain thresholds only when sufficient GABAergic activity is present.

Pentothal Barbiturates Muscimol Spinal cord Analgesia

BARBITURATES have been used as sedatives and anesthetics for many years, but their precise mechanism of action remains unclear (19,28). In vitro studies have shown that barbiturates can exert multiple effects on neuronal functioning including membrane stabilization (23), inhibition of the response of neurons to excitatory amino acids (7), reduction of the quantal content of excitatory transmitter released (18) and modulation of the binding of some neurotransmitters or neuromodulators to their receptors [adenosine: (11), GABA: (9), benzodiazepines: (2,24)]. Recent pharmacological studies suggest that the sedative effects of barbiturates are due to increased GABAergic activity (1,22). On the other hand, it is considered that the general anesthetic activity of barbiturates cannot be explained solely by enhancement of GABA actions, but most likely also involves the inhibition of excitatory neurotransmitter release (19).

Traditionally, barbiturates have been administered orally or systemically and are considered to be devoid of analgesic actions (6). Recently, however, there have been reports that barbiturates can produce analgesia when administered intrathecally at the spinal cord (5,25) as demonstrated by the prolongation of tail-flick latency (TFL) to radiant heat, a response indicative of analgesia but confounded with motor inhibition. It has been suggested that the analgesia produced by the intrathecal injection of pentobarbital is mediated through GABA activity, a conclusion based on the finding that large doses of the $GABA_A$ antagonists picrotoxin or bicuculline (12 μg and 25 μg respectively) interfered with the effect of pentobarbital on TFL (25). However, it has been reported that as little as 1 μg of bicuculline or picrotoxin administered intrathecally can elicit hyperalgesia and doses as low as 5 μg produce clonic and locomotor seizures in nonspinal-transected rats (20). Therefore, conclusions concerning the role of GABAergic activity in pentobarbital-induced analgesia may have been confounded by the use of large doses of the GABA antagonists. Moreover, enhancement of GABA binding by pentobarbital can be antagonized by picrotoxin but not by bicuculline (27). Therefore, in the present work, we decided to test further the hypothesis that the analgesic action of barbiturates is mediated through GABA by exploring the pharmacological interactions of pentothal with a subanalgesic dose of the $GABA_A$ agonist muscimol, using two nociceptive tests; tail-flick latency (TFL) and vocalization-threshold-to-tail-shock (VTTS), the latter of which is not confounded with motor inhibition produced by intrathecal drug administration. We report here that pentothal administered intrathecally does not result in analgesia unless administered in combination with a nonanalgesic dose of muscimol, in which case a significant and prolonged analgesia is produced.

METHOD

Experiments were conducted with adult female Sprague-Dawley rats housed individually at 23°C and maintained on a reverse day-night cycle (lights on: 20:00 to 10:00). Food and water were provided ad lib. All rats were ovariectomized under ketamine anesthesia (0.1 mg/kg) and allowed to recover for at least 2 weeks before implantation of an intrathecal catheter. A catheter (Clay Adams PE-10 tubing; Fisher Chemical, Springfield, NJ; 7.5 cm insertion length) was implanted chronically in the subarachnoid intrathecal space, extending to the lumbar level of the spinal cord (29). Testing did not begin for at least two weeks postimplantation. At the completion of the experiment the animals were over-anesthetized with Chloropent, perfused with 4% formalin and the spinal cord was dissected to verify correct placement of the catheter.

Vocalization-threshold-to-tail-shock (VTTS) was determined

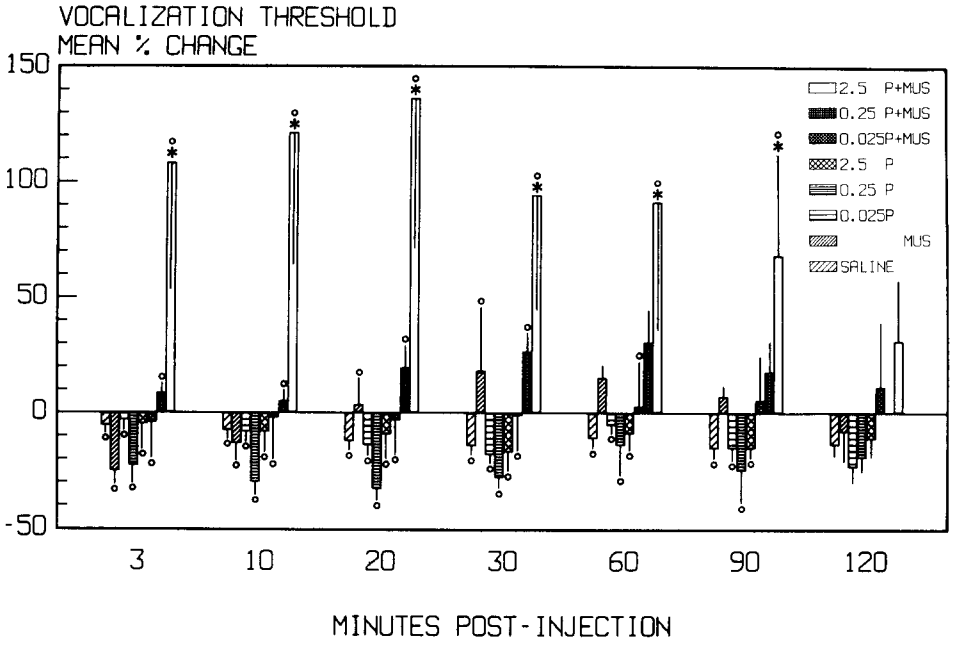

FIG. 1. Group mean percent changes (±S.E.M.) in vocalization-threshold-to-tail-shock (VTTS) after intrathecal injection as compared to preinjection values. Significant differences between drug treatments (based on Duncan's Multiple Range Test) at each time point postinjection are represented as follows: the value of any treatment in which an open circle (○) is accompanied by an asterisk is significantly different ($p<0.05$) from any other treatment with an open circle that is not accompanied by an asterisk at that time point only. Example: at 60 min "2.5 mg pentothal + 1 μg muscimol" is significantly different from "saline" but is not different from "1 μg muscimol." In this and the following figure, in all cases the dose of muscimol was 1 μg and the dose of pentothal is given in mg.

by placing rats in a Plexiglas restrainer and taping two stainless steel electrodes to the tail after applying conductive gel. Electrical shock (100-msec train of 60-Hz symmetrical, biphasic square waves) with an intertrain interval of 3 sec, were delivered by a constant current shock generator (Coulbourn Instruments Programmable Shocker, Lehigh Valley, PA). The current was increased stepwise in 10-amp units until vocalization was elicited ("upper shock level") and then decreased until vocalization ceased ("lower shock level"). This was repeated three times. Upper and lower shock levels were averaged to provide an estimate of vocalization threshold. Each female was pretested twice and the second test was used as the basis for calculating percent change postinjection.

Tail-flick latency (TFL) was determined with an IITC Model 33 Analgesimeter (Woodland Hills, CA; at 90% intensity). Rats were placed in a Plexiglas restrainer and the tail was exposed to a radiant heat lamp. TFL was measured automatically by activation of a photocell upon tail withdrawal. Each test score was the average of three trials. A cutoff after 10 sec maximum duration of exposure to the radiant heat was employed to avoid tissue damage.

After baseline testing, rats were injected intrathecally with muscimol (1 μg in 5 μl saline; Sigma Chemical, St. Louis, MO), a dose already found to be nonanalgesic (20), pentothal (high dose = 2.5 mg, intermediate dose = 0.25 mg or a low dose = 0.025 mg in 5 μl saline; Abbott Labs., Chicago, IL) or a combination of both drugs injected simultaneously. Controls received 5 μl saline. Postinjection tests began 3 min after injection. Statistical significance between groups was determined by Duncan's Multiple Range Test performed using a SAS computer program.

RESULTS

Effects on Vocalization-Threshold-to-Tail-Shock (VTTS)

Animals receiving combined administration of 1 μg muscimol and 2.5 mg pentothal intrathecally displayed a significantly greater percent increase in VTTS than animals rceiving any other drug or saline treatment at 3, 10, 20 and 30 min postinjection ($p<0.05$; Duncan's Multiple Range Test). At 60 and 90 min postinjection the animals receiving this combined treatment still had significantly greater VTTS than animals receiving saline or 2.5 mg pentothal alone, but were not different from those receiving 1 μg muscimol alone. VTTS values in the 1 μg muscimol-only group did not differ significantly from those in the saline or any pentothal-only group at any time postinjection. By 120 min postinjection all of the drug treatment groups did not differ significantly from each other ($p>0.05$; Duncan's Multiple Range Test; Fig. 1).

Effects on Tail-Flick Latency (TFL)

TFL values were significantly greater in the group receiving a combination of 1 μg muscimol and 2.5 mg pentothal intrathecally than in the groups receiving saline or 2.5 mg pentothal-only at 10, 20, 30, 60 and 90 min postinjection and significantly greater than in the groups receiving 1 μg muscimol-only at 30 and 60 min postinjection. TFL values were significantly greater in the 1 μg muscimol + 0.25 mg pentothal group than the 0.25 mg pentothal-only group at 20 and 60 min postinjection and significantly greater than the saline and 1 μg muscimol-only group at 60 min

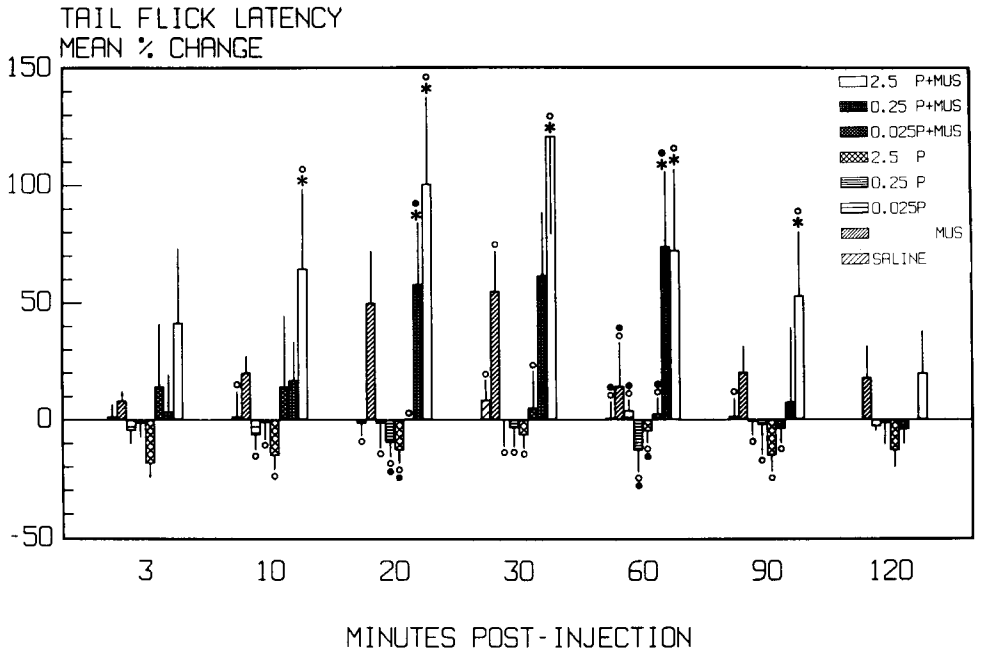

FIG. 2. Group mean percent changes (±S.E.M.) in tail-flick latency (TFL) after intrathecal injection as compared to preinjection values. Significant differences ($p<0.05$) between drug treatments (based on Duncan's Multiple Range Test) at each time point postinjection are represented as follows: the value of any treatment in which an open circle (○) is accompanied by an asterisk is significantly different from any other treatment with the same symbol that is not accompanied by an asterisk at that time point only, and likewise for treatments with closed circles (●). Example: at 20 min postinjection, "0.25 mg pentothal + 1 μg muscimol" and "2.5 mg pentothal + 1 μg muscimol" are not significantly different from each other or from "1 μg muscimol" but they are both different from "0.25 mg and 2.5 mg pentothal."

postinjection ($p<0.05$; Duncan's Multiple Range Test). In the 1 μg muscimol-only group, the TFL values were never significantly different from those in the saline or any pentothal-only groups. None of the treatment groups differed significantly from the others at 3 or 120 min postinjection ($p>0.05$; Duncan's Multiple Range Test; Fig. 2).

DISCUSSION

In the present experiment, the short-acting barbiturate pentothal administered alone at the doses employed failed to produce analgesia. Therefore, we found no evidence of a direct effect of barbiturate on analgesia. On the other hand, the present data demonstrate that the presence of a $GABA_A$ agonist exerts a permissive effect on the ability of pentothal to induce analgesia at the spinal cord. This suggests that the barbiturate amplified the effect of muscimol on GABAergic sites that suppress activity in the spinal pain pathways.

Electrophysiological and biochemical evidence that barbiturates enhance GABA activity is abundant (9). Thus, significant enhancement of GABA agonist binding occurs at and below intravenous anesthetic concentrations of pentobarbital (2,3). Barbiturates have also been reported to prolong the duration of GABA-induced postsynaptic opening of chloride channels (8,22) and prevent picrotoxin from antagonizing GABA-stimulated chloride flux (1). Moreover, it has been reported that at higher concentrations, barbiturates directly hyperpolarize motoneurons in a manner similar to GABA (14, 15, 26). Traditionally, barbiturates have been considered to not alter pain thresholds. Recently, however, there have been reports indicating that barbiturates may produce analgesia when administered intrathecally to the spinal cord, as demonstrated by the tail-flick test (5,25). However, in the present study we failed to find any increase in nociceptive thresholds in either the TFL or VTTS test after intrathecal pentothal. Furthermore, when nonanalgesia-producing doses of pentothal were combined with a nonanalgesia-producing dose of muscimol, significant and prolonged analgesia resulted. Thus, it appears that occupation of postsynaptic receptors by GABA or GABA agonists is essential for barbiturate-produced analgesia.

The level of occupation of GABA receptors by agonist may explain the variable results reported in the literature concerning the ability of barbiturates to alter pain perception. Failure of barbiturates to produce analgesia may be due to the absence or reduced rate of release of GABA at postsynaptic terminals at the time of testing. This condition of reduced GABAergic tone may be produced by the barbiturate itself through suppression of excitatory input to GABAergic neurons (21). This antagonistic action of barbiturates on analgesia, however, would be overcome when GABA or GABA agonists are exogenously administered in combination with the barbiturates, as in the present study. Within this context, it is interesting that intrathecal pentobarbital administration increases tail-flick latency more in spinal-transected animals than in intact rats (25). There is considerable evidence that powerful inhibitory influences are exerted from higher brain levels on inhibitory spinal interneurons (12). Perhaps spinal transection of descending pathways would disinhibit GABAergic interneurons, thereby increasing their activity and allowing barbiturates to facilitate analgesia in these animals.

The present results support the existence of a significant GABAergic system controlling afferent activity in the spinal

nociceptive pathway, possibly through GABAergic interneurons. High concentrations of GABA have been localized immunocytochemically in the dorsal horn of the spinal cord, and ultrastructural studies have revealed its presence both at axoaxonal and axosomatic synapses, a finding supporting the role of GABA in both presynaptic and postsynaptic inhibition (13). The fact that pentothal interacted with this GABAergic system to produce analgesia indicates that the GABA receptors that exist in the sensory system of the rat spinal cord possess a barbiturate-sensitive regulatory site that may function to modulate pain perception.

ACKNOWLEDGEMENTS

This work is contribution No. 481 from the Institute of Animal Behavior. The authors would like to thank Ms. Cynthia Banas for her excellent technical assistance.

REFERENCES

1. Allan, A. M.; Harris, R. A. Anesthetic and convulsant barbiturates alter gamma-aminobutyric acid-stimulated chloride flux across brain membranes. J. Pharmacol. Exp. Ther. 238:763–768; 1986.
2. Asano, T.; Ogasawara, N. Chloride-dependent stimulation of GABA and benzodiazepine receptor binding by pentobarbital. Brain Res. 225:212–216; 1981.
3. Asano, T.; Ogasawara, N. Stimulation of GABA receptor binding by barbiturates. Eur. J. Pharmacol. 77:355–357; 1982.
4. Barker, J. L. CNS depressants: effects on post-synaptic pharmacology. Brain Res. 92:35–55; 1975.
5. Carlsson, K.-H.; Jurna, I. Interaction of pentobarbital and morphine in the tail-flick test performed on rats: synergism at the spinal and antagonism at the supraspinal level. Neurosci. Lett. 71:356–360; 1986.
6. Clutton-Brock, J. Pain and the barbiturates. Anesthesia 16:80–88; 1961.
7. Collins, G. G.; Anson, J. Effects of barbiturates on responses evoked by excitatory amino acids in slices of rat olfactory cortex. Neuropharmacology 26:167–171; 1987.
8. Evans, R. H. Potentiation of the effects of GABA by pentobarbitone. Brain Res. 171:113–120; 1979.
9. Johnston, G. A. Regulation of GABA receptors by barbiturates and by related sedative-hypnotic and anticonvulsant drugs. In: Enna, S. J., ed. The GABA receptors. Clifton, NJ: The Humana Press; 1981: 107–128.
10. Keane, P. E.; Biziere, K. The effects of general anesthetics on GABAergic synaptic transmission. Life Sci. 41:1437–1448; 1987.
11. Loshe, M. J. Interaction of barbiturates with adenosine receptors in rat brain. Naunyn Schmiedebergs Arch. Pharmacol. 326:69–74; 1984.
12. Lundberg, A. Control of spinal mechanisms from the brain. In: Tower, D. B., ed. The nervous system, vol I: The basic neurosciences. New York: Raven Press; 1975:253–265.
13. Magoul, R.; Onteniente, B.; Geffard, M.; Calas, A. Anatomical distribution and ultrastructural organization of the GABAergic system in the rat spinal cord. An immunocytochemical study using anti-GABA antibodies. Neuroscience 20:1001–1009; 1987.
14. Nicoll, R. A. Pentobarbital: action on frog motoneurons. Brain Res. 96:119–123; 1975.
15. Nicoll, R. A.; Wojtowics, J. M. The effects of pentobarbital and related compounds on frog motoneurons. Brain Res. 191:225–237; 1980.
16. Olsen, R. W. GABA-benzodiazepine-barbiturate interactions. J. Neurochem. 37:1–13; 1981.
17. Ossipov, M. H.; Gebhart, G. F. Light pentobarbital anesthesia diminishes the antinociceptive potency of morphine administered intracranially but not intrathecally in the rat. Eur. J. Pharmacol. 97:137–140; 1984.
18. Richard, C. D. On the mechanism of barbiturate anaesthesia. J. Physiol. 227:749–767; 1972.
19. Richter, J. A.; Holtman, J. R., Jr. Barbiturates: their in vivo effects and potential biochemical mechanisms. Prog. Neurobiol. 18:275–319; 1982.
20. Roberts, L. A.; Beyer, C.; Komisaruk, B. R. Nociceptive responses to altered GABAergic activity at the spinal cord. Life Sci. 39: 1667–1674; 1986.
21. Sawada, S. Blocking actions of pentobarbital on receptors for excitatory amino acids in guinea pig hippocampus. Exp. Brain Res. 9:226–231; 1985.
22. Schulz, D. W.; MacDonald, R. L. Barbiturate enhancement of GABA mediated inhibition and activation of chloride ion conductance: correlation with anticonvulsant actions. Brain Res. 209:177–188; 1981.
23. Seeman, P. The membrane action of anesthetic tranquilizers. Pharmacol. Rev. 24:583–654; 1972.
24. Skolnick, P.; Rice, K. C.; Barker, J. L.; Paul, S. M. Interaction of barbiturates with benzodiazepine receptors in the central nervous system. Brain Res. 233:143–156; 1982.
25. Stein, C.; Morgan, M. M.; Liebeskind, J. C. Barbiturate induced inhibition of a spinal nociceptive reflex: role of GABA mechanisms and descending modulation. Brain Res. 407:307–311; 1987.
26. Whitney, J. F.; Glenn, L. L. Pentobarbital and halothane hyperpolarize cat α-motoneurons. Brain Res. 381:199–203; 1986.
27. Willow, M. A comparison of the actions of pentobarbitone and etomidate on [^{3}H]GABA binding to crude synpatosomal rat brain membranes. Brain Res. 220:427–431; 1981.
28. Willow, M. A.; Johnston, G. A. Pharmacology of barbiturates: electrophysiological and neurochemical studies. Int. Rev. Neurobiol. 24:15–49; 1983
29. Yaksh, T. L.; Rudy, T. A. Chronic catheterization of the subarachnoid space. Physiol. Behav. 17:1031–1036; 1976.