Pharmacology Biochemistry & Behavior, Vol. 16, pp. 737–739, 1982. Printed in the U.S.A.

Rotation induced by Intranigral Phenobarbital: Evidence of Barbiturate GABAergic Activity

JOSEPH T. McDEVITT AND LIBBY M. YUNGER

Department of Pharmacology, Smith Kline & French Laboratories, Philadelphia, PA 19101

Received 16 November 1981

McDEVITT, J. T. AND L. M. YUNGER. *Rotation induced by intranigral phenobarbital: Evidence of barbiturate GABAergic activity.* PHARMAC. BIOCHEM. BEHAV. 16(5) 737–739, 1982.—Phenobarbital, injected unilaterally into the pars reticulata of the substantia nigra, induced significant contraversive rotation. Similar to the rotation induced by intranigral administration of GABA agonists, phenobarbital-induced rotation was antagonized by picrotoxin and persisted in animals with 6-hydroxydopamine-induced denervation of the ipsilateral nigrostriatal dopamine system. The results of this study support the hypothesis that the anticonvulsant barbiturates enhance central GABA-mediated events.

Phenobarbital Barbiturates Substantia nigra Circling behavior GABA

RECENT electrophysiological and biochemical reports have demonstrated that barbiturates augment GABA-mediated neuronal inhibition [3, 4, 8]. This GABAergic effect caused by barbiturates may account, at least in part, for the anticonvulsant and/or anesthetic activity produced by this class of compounds. However, there is a paucity of behavioral data demonstrating that the barbiturates can augment or minic the effects of GABA in the central nervous system.

Contraversive rotation produced by unilateral administration of GABA agonists into the pars reticulata of the substantia nigra (SNR) is a well documented behavioral effect demonstrating enhanced central GABAergic activity [1, 2, 9, 12, 13]. Although contraversive rotation is also produced by opiates, glycine and substance P under similar experimental conditions, pharmacological and regional distinctions have allowed investigators to differentiate GABA-induced rotation from that caused by other agents [1, 6, 7]. Our results demonstrate, that when injected unilaterally into SNR, phenobarbital produced contraversive rotation which was pharmacologically and regionally similar to the rotational effects of GABA agonists in this procedure.

METHOD

Male albino rats obtained from Charles River Farms weighing 250 to 300 g were anesthetized with 10 mg/kg IV, of sodium brevital and secured into a stereotaxic frame. The scalp was incised, a burr hole drilled in the skull and a 33 ga needle was lowered into SNR. Injection coordinates were calculated using the DeGroot stereotaxic atlas [5]. Test substances were dissolved in sterile distilled H_2O and infused in a volume of 0.5 μl, at a rate of 0.5 μl/min. Immediately following removal of the injection needle and closure of the scalp, the animals were placed into hemispherical test cages where they awoke from anesthesia. The time to awakening was 7 (6–10) min; there was no evidence that any of the treatments potentiated the anesthetic. The number of 360° turns were counted automatically for each 3 minute interval for two hours.

Animals were examined histologically for the location of the injection site as follows. Brains were removed, mounted sagittally, frozen, and sectioned. The injection tract was located and the distance from the most anterior point of SNR, visible using 10x magnification, to the most ventral aspect of the injection tract was measured. Sites located 0.0 to 0.9, 1.0 to 1.8 and 1.9 to 2.7 mm were labeled rostral, middle and caudal SNR respectively.

Unilateral lesions of the ascending nigro-striatal pathway were induced by intracerebral injection of 8 μg 6-hydroxydopamine/4 μl vehicle [10]. The lesions were verified by the ability of apomorphine (0.5 mg/kg, IP) to induce greater than 200 contraversive turns per hour.

The data were analyzed by Student's *t*-test, two-tailed.

Sodium brevital was obtained from Eli Lilly (Indianapolis, IN), GABA was obtained from Sigma Chemical Co. (St. Louis, MO) and 6-hydroxydopamine was obtained from Regis Chemical Co. (Chicago, IL).

RESULTS

As illustrated in Fig. 1, phenobarbital produced contraversive rotation when injected unilaterally into SNR. Contraversive rotation was observed after either rostral or caudal SNR injections and over a range of doses from 12.5 to 100.0 μg. Compared to the GABA agonist muscimol, phenobarbital's duration of action in this procedure was relatively short (see Fig. 2). Similarities between the onset and duration of turning caused by GABA and phenobarbital, however, were observed. SNR injections of procaine (10

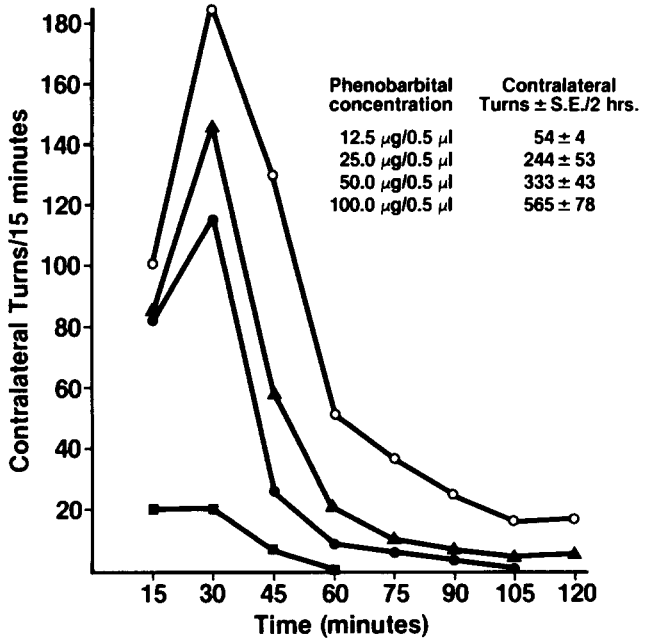

FIG. 1. Contraversive rotation induced by phenobarbital injected into SNR; ■ (12.5 μg/0.5 μl), ● (25.0 μg/0.5 μl), ▲ (50.0 μg/0.5 μl), and ○ (100.0 μg/0.5 μl), n=6, 8, 10 and 9 respectively.

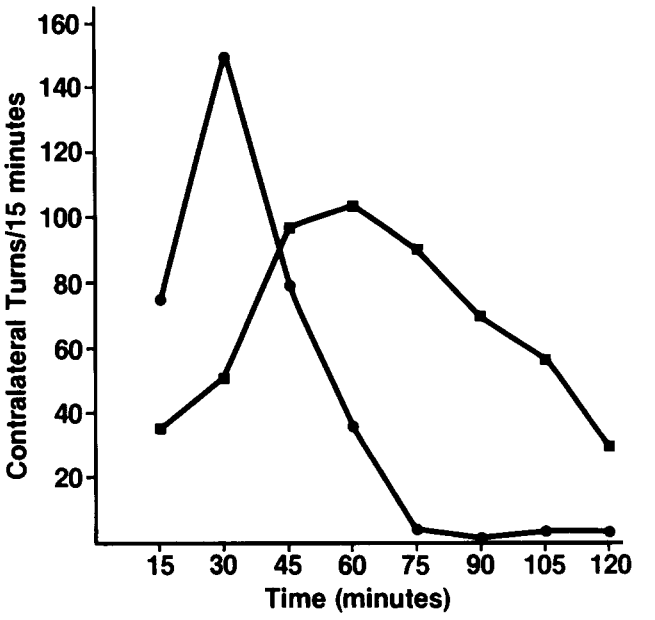

FIG. 2. Contraversive rotation induced by GABA, ●, 100 μg/0.5, μl (n=9), and muscimol, ■, 6.3 ng/0.5 μl (n=5).

μg/0.5 μl) or physiological saline (0.5 μl) were without effect, as were injections of phenobarbital (50.0 μg) into substantia nigra pars compacta.

The GABA antagonist picrotoxin (1.8 mg/kg IP) significantly attenuated the contraversive rotation caused by SNR injections of phenobarbital and GABA, but had no effect on glycine induced turning (Table 1). Conversely, the glycine antagonist strychnine (0.5 mg/kg, IP) significantly reduced the contraversive rotation caused by SNR injections of glycine, but had no effect on GABA or phenobarbital induced turning. These subconvulsant doses of picrotoxin and strychnine produced no overt behavioral effects in the rats.

Finally, when phenobarbital (25.0 μg) was injected unilaterally into the SNR of animals with prior 6-hydroxydopamine-induced unilateral lesions [10] of the substantia nigra, it produced 201±12 (n=6) contraversive turns in 2 hrs. This was not significantly different from the effect of 25.0 μg phenobarbital (i.e., 244±53 turns/2 hrs) in the intact preparation.

TABLE 1

EFFECT OF PICROTOXIN* (1.8 mg/kg, IP) OR STRYCHNINE* (0.5 mg/kg, IP) ON GABA, PHENOBARBITAL OR GLYCINE-INDUCED SNR ROTATION

Agonist	Antagonist	n	Contraversive Turns/2 hrs mean ± SE
GABA 150 μg	Saline	9	307 ± 39
GABA 150 μg	Picrotoxin	7	89 ± 23†
GABA 150 μg	Strychnine	5	411 ± 105
Phenobarbital 100 μg	Saline	9	565 ± 78
Phenobarbital 100 μg	Picrotoxin	8	188 ± 21†
Phenobarbital 100 μg	Strychnine	6	441 ± 29
Glycine 50 μg	Saline	13	536 ± 87
Glycine 50 μg	Picrotoxin	8	532 ± 106
Glycine 50 μg	Strychnine	7	91 ± 22†

*Administered immediately prior to agonist.

†$p<0.01$ different from saline treated group, Student's t-test, two-tailed.

DISCUSSION

One pharmacological characterization of GABA-mediated behaviors, including turning behavior, is antagonism by picrotoxin or bicuculline. Furthermore, GABA-mediated turning persists in animals with 6-hydroxydopamine-induced denervation of the ipsilateral nigrostriatal dopamine system [1,13]. Since rotation induced by phenobarbital injected into SNR was antagonized by picrotoxin and persistent in the 6-hydroxydopamine-lesioned animal, it is consistent with a GABAergic effect. Furthermore, phenobarbital-induced rotation, like GABA-induced but unlike glycine-induced rotation, was not reduced by strychnine.

Regional differences among the classes of compounds which induce rotation after intranigral injection have been noted. GABA-agonists, unlike substance P or opiates, do not cause ipsiversive turning when injected into any SNR region, nor do GABA agonists produce rotation when injected into the parts compacta of the substantia nigra [1,13]. Likewise phenobarbital produced only contraversive rotation when in-

jected into either the caudal or the rostral SNR and had no rotational effect when injected into the pars compacta.

In order to address the specficity of phenobarbital-induced rotation and to determine the extent to which local anesthetic properties of barbiturates may have been involved, a local anesthetic concentration of procaine was also injected into SNR. The ineffectiveness of procaine in this test system suggested that the rotational effect of phenobarbital was not due to a non-specific membrane effect.

Barbiturates and picrotoxin bind to the ionophore site (Cl^- channel) associated with the GABA receptor, and this binding apparently facilitates the Cl^- conductance changes associated with GABAergic neurotransmission [8,11]. However, unlike phenobarbital, anesthetic barbiturates also increase Cl^- conductance directly, producing membrane hyperpolarization. While all GABA receptors appear to be associated with Cl^- channels there may be more than one class of associated ionophore sites. Furthermore, the converse has not been demonstrated, that is, that all Cl^- ionophore sites (picrotoxinin binding sites) are linked to GABA receptors. In fact, there is evidence of heterogeneity of picrotoxinin binding sites [11]. Thus, the behavioral and electrophysiological effects of the two classes of barbiturates may reflect differences in the populations of sites bound, differences in the consequences of binding at the GABA-receptor-linked site, or some other mechanism. As a result, one cannot necessarily predict that both classes of barbiturates will produce rotation.

It should also be noted that all of the rats received sodium brevital, a rapidly metabolized anesthetic barbiturate. While its half-life in brain is estimated to be only 15–30 minutes, residual barbiturate might be expected to produce a slight priming effect on responses mediated via GABA receptor activation or increased Cl^- conductance. However, since the anesthetic was common to all animals, and the drug presumably affected both sides of the brain equally, it can be defined as a property of the baseline condition of the experimental animals.

In conclusion, the contraversive rotation caused by phenobarbital in our study appeared to be GABAergic in origin. Similarities between the rotation caused by phenobarbital and GABA itself suggests that phenobarbital may be acting via a direct effect on endogenous GABA within SNR. Our data, therefore, support electrophysiological and biochemical reports that anticonvulsant barbiturates augment central GABAergic mechanisms.

REFERENCES

1. Arnt, J. and J. Scheel-Kruger. GABAergic and glycinergic mechanisms within the substantia nigra: Pharmacological specificity of dopamine-independent contralateral turning behavior and interactions with other neurotransmitters. *Psychopharmacology* **62:** 267–277, 1979.
2. Arnt, J., J. Scheel-Kruger, G. Magelund and P. Krogsgaard-Larsen. Muscimol and related GABA receptor agonists: The potency of GABAergic drugs in vivo determined after intranigral injection. *J. Pharm. Pharmac.* **31:** 306–313, 1979.
3. Barker, J. L., J. F. MacDonald and D. A. Mathers. Three GABA receptor functions on mouse spinal neurons. *Brain Res. Bull.* **5:** Suppl. 2, 43–49, 1980.
4. Costa, E. and A. Guidotti. In: *GABA in Nervous System Function*, edited by E. Roberts, T. N. Chase and D. B. Tower. New York: Raven Press, 1976, pp. 413–426.
5. DeGroot, J. The rat forebrain in stereotaxic coordinates. *Proc. K. ned. Akad. Wet., C* **52:** 1–40, 1959.
6. Iwamoto, E. T. and E. L. Way. Circling behavior and stereotypy induced by intranigral opiate microinjections. *J. Pharmac. exp. Ther.* **203:** 347–359, 1977.
7. James, T. A. and M. S. Starr. Behavioral and biochemical effects of substance P injected into the substantia nigra of the rat. *J. Pharm. Pharmac.* **29:** 181–182, 1977.
8. MacDonald, R. L. and J. L. Barker. Different actions of anticonvulsant and anesthetic barbiturates revealed by use of cultured mammalian neurons. *Science* **200:** 775–777, 1978.
9. Olpe, H. R., H. Schellenberg and W. P. Koella. Rotational behavior induced in rats by intranigral application of GABA-related drugs and GABA-antagonists. *Eur. J. Pharmac.* **45:** 291–294, 1977.
10. Setler, P. E., M. Malesky, J. McDevitt and K. Turner. Rotation produced by administration of dopamine and related substances directly into the supersensitive caudate nucleus. *Life Sci.* **23:** 1277–1284, 1978.
11. Ticku, M. K. and R. W. Olsen. Interaction of barbiturates with dihydropicrotoxinin binding sites related to the GABA receptor-ionophore system. *Life Sci.* **22:** 1643–1652, 1978.
12. Waddington, J. L. Induction of rotational behavior by intranigral baclofen suggest possible GABA agonist activity. *Experientia* **33:** 1345, 1977.
13. Waddington, J. L. Behavioral evidence for GABAergic activity of the benzodiazepine flurazepam. *Eur. J. Pharmac.* **51:** 417–422, 1978.