Mechanism of Action of Anticonvulsant Drugs III. Chlordiazepoxide

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The anticonvulsant activity of chlordiazepoxide in mice was found to be antagonized by pretreatment with reserpine or Ro4-1284 but not by α-methyl dopa or α-methyl tyrosine. This antagonism of chlordiazepoxide by reserpine was reversed by the administration of α-methyl dopa, *d*-amphetamine, or 5-hydroxytryptophan. The failure of α-methyl tyrosine to antagonize chlordiazepoxide suggests that the anticonvulsant action of chlordiazepoxide is not mediated through the release of biogenic amines. Similarly, the reserpine antagonism of chlordiazepoxide appears to be mediated through some mechanism other than catecholamine depletion.

EARLIER communications from these laboratories have described the effects of various amine-depleting agents on the anticonvulsant properties of diphenylhydantoin (1) and acetazolamide (2). These reports have confirmed the findings of earlier workers (3, 4) that reserpine antagonizes the anticonvulsant activities of both diphenylhydantoin and acetazolamide. In addition, however, the authors have demonstrated that this action is not specific for reserpine, but that the benzoquinolizine derivatives, tetrabenazine and Ro4-1284[1] also antagonize both anticonvulsants. Furthermore, acetazolamide was found to be antagonized by all agents which deplete brain biogenic amines, whereas diphenylhydantoin was not. These results suggest that the anticonvulsant action of acetazolamide is mediated, in some manner, through brain amines. The action of diphenylhydantoin is probably not mediated through brain amine release.

The experiments reported in this communication were conducted to compare the action of chlordiazepoxide, which has been reported by Randall *et al.* (5) to possess anticonvulsant activity in mice, to the anticonvulsant activities of acetazolamide and diphenylhydantoin.

EXPERIMENTAL

Male albino mice (Harlan Industries) weighing 18–22 Gm. were used in all experiments. Each animal was used only once. Prior to experimentation the mice were housed in groups of 50 with free access to food and water. All drugs were administered intraperitoneally and doses and pretreatment times are shown in Tables I and II.

Maximal seizures were produced by the method of Swinyard *et al.* (6) employing a current of 50 ma. and 0.2-sec. duration delivered *via* corneal electrodes. The criterion for protection against maximal electroshock was abolition of the hind leg extensor component of the seizure.

The anticonvulsant potency of chlordiazepoxide was compared in groups of mice treated with various amine-depleting agents by determining the dose of chlordiazepoxide which protected 50% of the mice against the electroshock. The ED_{50} values were estimated and compared for significance of differences by the method of Litchfield and Wilcoxon (7).

Chlordiazepoxide was always the last drug to be administered and was followed in 30 min. by electroshock. Unless otherwise stated, mice were housed in individual plastic cages prior to electroshock. In one experiment the animals were aggregated, in groups of 10, in stainless steel cages.

RESULTS

The effects of the various amine-depleting agents on the ED_{50} value of chlordiazepoxide in the maximal electroshock test are shown in Table I. Both reserpine, at 5.0 mg./Kg., and Ro4-1284, at 100 mg./Kg., antagonized the anticonvulsant effect of chlordiazepoxide as evidenced by a significant increase in the ED_{50} value ($p < 0.05$). Neither α-methyl tyrosine nor α-methyl dopa, at doses of 400 mg./Kg., altered the ED_{50} value of chlordiazepoxide.

TABLE I.—EFFECTS OF AMINE-DEPLETING AGENTS ON THE ANTICONVULSANT POTENCY OF CHLORDIAZEPOXIDE

Treatment	i.p. Dose, mg./Kg.	Pretreatment Time, hr.	ED_{50} of Chlordiazepoxide,[a] mg./Kg.
Saline	...	4	23(19–28)
Reserpine	5.0	24	88(64–121)[b]
Ro4-1284	100	4	66(58–75)[b]
α-Methyl tyrosine	400	4	19(16–22)
α-Methyl dopa	400	4	22(18–26)

[a] 95% confidence limits shown in parenthesis. [b] Significantly different from saline controls ($p < 0.05$).

In order to test the ability of various agents to reverse the effects of reserpine on the anticonvulsant potency of chlordiazepoxide, a series of experiments were performed in which reserpinized mice (5.0 mg./Kg.) received a single dose of the test compound 1 hr. prior to electroshock. This was followed in 30 min. by varying doses of chlordiazepoxide. The ED_{50} values for chlordiazepoxide, in combination with the various compounds, were determined 30 min. later. The results of this experiment are shown in Table II. α-Methyl dopa (400 mg./Kg.), *d*-amphetamine (5.0 mg./Kg.), and 5-hydroxytryptophan (500 mg./Kg.), when administered 30 min. prior to chlordiazepoxide, significantly reduced the ED_{50} value of chlordiazepoxide in reserpinized mice ($p < 0.05$). The 400 mg./Kg. dose of dopa, when administered to reserpinized mice in the isolated situation failed to alter the ED_{50} value of chlordiazepoxide. When this dose of dopa was administered to aggregated mice, however, a significant reduction in the ED_{50} value was produced ($p < 0.05$).

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[1] 2-Hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy 1,2,3,4,6,7-hexahydro-11bH-benzoquinolizine.

TABLE II.—ANTAGONISM OF RESERPINE EFFECT ON THE ANTICONVULSANT POTENCY OF CHLORDIAZEPOXIDE

Treatment	i.p. Dose, mg./Kg.	Pretreatment Time, hr.	ED_{50} of Chlordiazepoxide,[a] mg./Kg.
Reserpine	5.0	24	88(64–121)
Saline	5.0	1	
Reserpine	5.0	24	34(29–40)[b]
α-Methyl dopa	400	1	
Reserpine	5.0	24	52(43–64)[b]
d-Amphetamine	5.0	1	
Reserpine	5.0	24	53(45–63)[b]
5-OH Tryptophan	500	1	
Reserpine	5.0	24	71(56–90)
Dopa	400	1	
Reserpine	5.0	24	57(47–68)[b]
Dopa (aggregated)[c]	400	1	

[a] 95% confidence limits shown in parenthesis. [b] Significantly different from reserpine–saline controls ($p < 0.05$). [c] Housed 10/cage after administration of dopa.

DISCUSSION

The results of this study demonstrate that the effects of amine-depleting agents on the anticonvulsant properties of chlordiazepoxide more closely resemble the effects of similar interactions for diphenylhydantoin than for acetazolamide which have been reported earlier (1, 2). With respect to the effects of reserpine and Ro4-1284, chlordiazepoxide resembles both diphenylhydantoin and acetazolamide in that the anticonvulsant effects of all three of these agents are antagonized by these amine depletors. In the case of interaction with α-methyl dopa, chlordiazepoxide closely resembles diphenylhydantoin in that the anticonvulsant effects of both were unchanged by this amine depletor. The failure of amine depletion by α-methyl dopa to antagonize either diphenylhydantoin or chlordiazepoxide might be explained on the basis of the metabolic fate of α-methyl dopa. α-Methyl dopa is metabolized to α-methyl norepinephrine (8). This metabolite may serve as a false neurotransmittor, an effect which could prevent the antagonism of the anticonvulsants by amine depletion *per se*. This seems unlikely, however, since the authors have found that α-methyl dopa antagonizes the anticonvulsant action of acetazolamide (2). Also, amine depletion by α-methyl tyrosine, which is not metabolized to a catecholamine-like structure (9) is without effect on either diphenylhydantoin (1) or chlordiazepoxide but was found to antagonize acetazolamide (2).

Further similarities between chlordiazepoxide and diphenylhydantoin in these experiments are that the administration of 5-hydroxytryptophan, α-methyl dopa, or *d*-amphetamine to reserpinized mice antagonized the effect of reserpine on the anticonvulsant effects of both chlordiazepoxide and diphenylhydantoin. Since dopa failed to antagonize the reserpine effect in isolated mice the authors studied this interaction in aggregated animals. Dopa has been shown to antagonize the effect of reserpine on the anticonvulsant action of acetazolamide in aggregated but not isolated mice (2). This effect of aggregation may be due to an increased uptake of dopa into the central nervous system. It has been shown by other workers that amphetamine is concentrated in the central nervous system to a greater extent in aggregated than in isolated mice (10).

It appears that the mechanism of the antagonism of the anticonvulsant effect of chlordiazepoxide by reserpine and Ro4-1284 is probably not the result of amine depletion, nor does the anticonvulsant effect of chlordiazepoxide appear to be mediated through brain catecholamines since (*a*) neither of the amine depletors, α-methyl dopa nor α-methyl tyrosine, was effective in antagonizing chlordiazepoxide and (*b*) the reserpine antagonism of chlordiazepoxide was reversed by *d*-amphetamine. On the basis of the results of the present study as well as earlier work from these laboratories (1, 2) the anticonvulsant effect of chlordiazepoxide appears to resemble that of diphenylhydantoin rather than acetazolamide.

REFERENCES

(1) Rudzik, A. D., and Mennear, J. H., *Life Sciences*, **4**, 2373(1965).
(2) *Ibid.*, **5**, 747(1966).
(3) Chen, G., Ensor, C. R., and Bohner, B., *Proc. Soc. Exptl. Biol. Med.*, **86**, 507(1954).
(4) Gray, W. D., Rauh, C. E., Osterberg, A. C., and Lipchuck, L. M., *J. Pharmacol. Exptl. Therap.*, **124**, 149 (1958).
(5) Randall, L. O., Schallek, W., Heise, G. A., Keith, E. F., and Bagdon, R. E., *ibid.*, **129**, 163(1960).
(6) Swinyard, E. A., Brown, W. C., and Goodman, L. S., *ibid.*, **106**, 319(1952).
(7) Litchfield, S. T., and Wilcoxon, F., *ibid.*, **96**, 99 (1949).
(8) Carlson, A., and Lindqvist, M., *Acta Physiol. Scand.*, **54**, 87(1962).
(9) Spector, S., Sjoerdsma, A., and Udenfriend, S., *J. Pharmacol. Exptl. Therap.*, **147**, 86(1965).
(10) Consolo, S., Garattini, S., Ghielmetti, R., and Valzelli, L., *J. Pharm. Pharmacol.*, **17**, 666(1965).