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THE EFFECTS OF RESERPINE AND OTHER AGENTS UPON LEPTAZOL CONVULSIONS IN MICE

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Reserpine and tetrabenazine reduced the survival time of mice infused with leptazol. This effect was antagonized by pretreatment with iproniazid. The survival time of iproniazid-treated mice was prolonged by 5-hydroxytryptophan but not by 3:4-dihydroxyphenylalanine. These findings suggest a relation between leptazol sensitivity and brain levels of 5-hydroxytryptamine.

It has been shown by Chen and his co-workers (Chen, Ensor, and Bohner, 1954) that the survival time of mice infused intravenously with leptazol may be shortened by pretreatment with reserpine. Reserpine is unique in being the only sedative agent for which this property has been shown; barbiturates lengthen survival time (Chen *et al.*, 1954), while chlorpromazine has been reported to be without effect (Balestrieri, 1955). The sedative properties of reserpine have been attributed to the changes which this drug produces in brain levels of 5-hydroxytryptamine and catechol amines. We have therefore investigated the action of drugs altering brain amine metabolism and other agents upon the facilitation of leptazol by reserpine. After this work was completed, a paper appeared describing experiments similar to those reported below and reaching essentially the same conclusions (Kobinger, 1958).

METHOD

The method used by Chen *et al.* (1954) was that due to Orloff, Williams and Pfeiffer (1949). They injected mice intravenously with 0.05 ml. of a solution containing 0.25 mg. of leptazol every 10 sec. The volume injected between the first twitch and the terminal extensor spasm was the measure found to be reduced by reserpine; the volume injected before the first sign appeared was unaffected.

We have used a constant-rate injection machine for infusing into the tail vein a 0.5% leptazol solution containing a dye at the rate of 0.24 ml./min. LAC grey mice of either sex were used, weighing 16 to 23 g. We have preferred to measure the total period from commencing the infusion until the terminal convulsion occurred and this we have called the survival time. Drugs were injected intraperitoneally in volumes of 0.1 ml./10 g. of body weight.

RESULTS

The mean survival time of 139 mice infused with leptazol was 114.1 $\pm$ s.e. 2.8 sec. This value is derived from animals used, in groups of about 10, as controls in a number of experiments. The mean values for these groups ranged from 90 to 130 sec. with standard errors from ± 7 to ± 15 sec.

Rauwolfia Alkaloids

Reserpine.—The change in survival time of groups of mice infused with leptazol at various intervals after treatment with 2 mg./kg. of reserpine is shown in Fig. 1. It will be seen that no significant reduction occurred up to 1¼ hr. after reserpine ($P>0.05$), but that from 1¾ hr. survival time became shorter ($P<0.01$), reaching a minimum in about 4 hr. and was still slightly below normal 3 days after reserpine ($P<0.05$). The dose of reserpine used produced hypothermia and sedation in mice, lasting about 2 days. Their body temperatures measured at the end of this period, by a method previously described (Lessin and Parkes, 1957), had regained normal values ($37.2 \pm 0.2°$).

With doses of reserpine smaller than 2 mg./kg., no significant effect upon survival time could be shown.

Tetrabenazine

This compound, 3-*iso*butyl-1:2:3:4:6:7-hexahydro-9:10-dimethoxy-11bH-benzo[*a*]quinolizin-2-one (Pletscher, 1957a; Pletscher, Besendorf, and Bächtold, 1958), is a member of a group of synthetic compounds shown to reduce brain amine levels. It causes sedative effects in

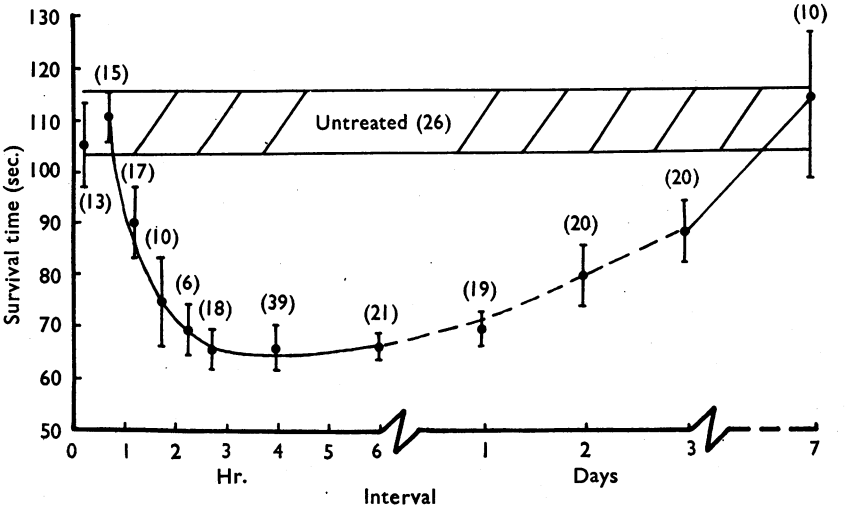

FIG. 1.—Leptazol survival time of groups of mice pretreated with 2 mg./kg. of reserpine intraperitoneally. Ordinate, survival time in sec. Abscissa, interval between reserpine injection and leptazol infusion. Each point is the mean for the group, with its standard error; the number of mice in the group is shown in brackets. The shaded area gives the limits between the mean value±s.e. for survival time of mice untreated with reserpine.

experimental animals resembling those of reserpine, although of shorter duration. Table I shows that this compound (75 mg./kg.) caused considerable reduction of survival time within 1½ hr.

Agents Affecting Amine Metabolism

Iproniazid.—Pretreatment with 200 mg./kg. of iproniazid did not affect the survival time of mice infused with leptazol 3 to 24 hr. later (Table I), but this treatment prevented the reduction of survival time by effective doses of reserpine injected 24 hr. later. Hertting (1958) has also demonstrated this antagonism. Tetrabenazine was also rendered ineffective by pretreatment with 200 mg./kg. of iproniazid (Table I). This dose of iproniazid is adequate for complete inhibition of brain monoamine oxidase in mice within 24 hr. (Davison, Lessin, and Parkes, 1957).

(±)-5-*Hydroxytryptophan.*—This agent, in doses of 50 mg./kg. injected 1 hr. before leptazol infusion, lengthened the survival time of mice that had been treated with iproniazid (200 mg./kg.) 24 hr. previously (Table I). When this pretreatment was omitted, 5-hydroxytryptophan did not alter survival time, even in a dose of 100 mg./kg., nor did it produce the tremors observed when 5-hydroxytryptophan was administered to iproniazid-treated mice.

(±)-3:4-*Dihydroxyphenylalanine.*—This substance (50 mg./kg.) given 45 min. before leptazol infusion produced no alteration in survival time of mice pretreated with iproniazid (Table I). Signs of excitement were observed in

TABLE I

EFFECT OF VARIOUS DRUGS UPON THE SURVIVAL TIME OF MICE INFUSED INTRAVENOUSLY WITH LEPTAZOL

The numerals in brackets give the number of animals used.

Treatment	Interval between Last Treatment and Leptazol	Mean Survival Time (Sec.±s.e.)	
Iproniazid, 200 mg./kg.	3 hr.	106·0±16·2 (6)	$P=0·5$
Control	—	122·6±11·7 (7)	
Reserpine, 2 mg./kg.	2 hr.	74·0± 9·0 (10)	$P<0·01$
Reserpine, 2 mg./kg., 24 hr. after iproniazid, 200 mg./kg.	2 ,,	117·3±15·4 (6)	$P<0·05$
Tetrabenazine, 75 mg./kg.	1½ hr.	44·1± 2·2 (11)	$P=0·001$
Control	—	94·4±12·4 (10)	
Iproniazid, 200 mg./kg.	24 hr.	117·7±11·8 (10)	
Tetrabenazine, 75 mg./kg., 24 hr. after iproniazid, 200 mg./kg.	1½ ,,	108·1±10·6 (12)	$P=0·5$
5-hydroxytryptophan, 50 mg./kg.	30 min.	103·8±10·0 (10)	$P=0·7$
Control	—	98·0± 8·7 (10)	
Iproniazid, 200 mg./kg.	24 hr.	95·9± 7·3 (10)	
5-Hydroxytryptophan, 50 mg./kg., 24 hr. after iproniazid, 200 mg./kg.	30 min.	148·7± 6·6 (12)	$P<0·001$
Dihydroxyphenylalanine, 100 mg./kg., 24 hr. after iproniazid, 200 mg./kg.	30 min.	93·1±11·6 (9)	
Control	—	106·5± 6·0 (8)	
Lysergic acid diethylamide, 2·5 mg./kg.	15 min.	119·7± 8·4 (12)	
Reserpine, 2 mg./kg.	4½ hr.	65·3± 7·1 (17)	
Lysergic acid diethylamide, 2·5 mg./kg., 4 hr. after reserpine, 2 mg./kg.	15 min.	93·2± 5·8 (12)	$P<0·01$
Reserpine, 2 mg./kg.	4½ hr.	55·9± 1·0 (8)	
2-Bromo-(+)-lysergic acid diethylamide, 2·5 mg./kg., 4 hr. after reserpine, 2 mg./kg.	15 min.	68·7± 8·6 (12)	P 0·1–0·2

these mice following the injection of dihydroxyphenylalanine similar to those reported by Carlsson, Lindquist, and Magnusson (1957).

Other Agents

(+)-Lysergic Acid Diethylamide. — Lysergic acid diethylamide (2.5 mg./kg.) antagonized the effect of reserpine on leptazol survival time when given 5 to 20 min. before the convulsant. This dose did not affect the survival time of normal mice (Table I).

2-Bromo-(+)-lysergic Acid Diethylamide

This compound shares with lysergic acid diethylamide the property of antagonizing the stimulant actions of 5-hydroxytryptamine upon smooth muscle, while lacking the centrally excitant properties of the parent compound (Cerletti and Rothlin, 1955). It was without action on the survival time of mice treated with reserpine under the same experimental conditions as those in which lysergic acid diethylamide showed antagonism to reserpine facilitation (Table I).

Discussion

Comparison of the time course of facilitation of leptazol convulsions with that of sedation and hypothermia by similar doses of reserpine in mice (Lessin and Parkes, 1957) showed that, whereas both types of effect were delayed in appearance for an hour or so after administration and reached their greatest intensity over the period 2 to 24 hr., they differed in duration. Survival time had not regained normal levels 3 days after reserpine, although body temperature had recovered within 2 days. A similar comparison may be drawn between the time course of leptazol facilitation by reserpine and the reduction in the levels of brain 5-hydroxytryptamine which this drug produced. This effect commenced without delay and was maximal 4 hr. after injection, while only partial recovery has occurred by 2 days in the mouse (Pletscher, personal communication) as in the rabbit (Pletscher, Shore, and Brodie, 1956). The similarity between the time courses of these two effects suggests that a relation may exist between leptazol facilitation and brain amine level similar to that postulated for the sedative properties of reserpine (Pletscher, Shore, and Brodie, 1956).

Further evidence for this relationship is provided by the action of iproniazid, since this agent, which has been shown to prevent the depletion of brain 5-hydroxytryptamine by reserpine (Besendorf and Pletscher, 1956; Pletscher, 1956), could also abolish the effect of reserpine upon leptazol survival time. In addition, the action of tetrabenazine, which both facilitated leptazol convulsions and reduced brain 5-hydroxytryptamine (Pletscher, 1957b; Pletscher, Besendorf, and Bächtold, 1958), effects which are prevented by iproniazid, lends further support to the argument.

Although treatment with iproniazid alone had no effect upon survival time, the subsequent administration of 5-hydroxytryptophan increased resistance to leptazol. This combination has been shown to raise brain levels of 5-hydroxytryptamine considerably (Udenfriend, Weissbach, and Bogdanski, 1957) and this may be seen as an extension of the relationship between sensitivity to leptazol and brain 5-hydroxytryptamine level. We were unable to demonstrate the protecting action of dihydroxyphenylalanine in iproniazid-treated mice reported by Kobinger (1958). Since administration of dihydroxyphenylalanine after iproniazid raised the level of brain catechol amines (Pletscher, 1957b), it may be suggested that these amines do not play a part in leptazol sensitivity. This contrasts with the situation regarding the sedative actions of reserpine, for which Carlsson *et al.* (1957) found dihydroxyphenylalanine a better antagonist than 5-hydroxytryptophan.

The action of lysergic acid diethylamide in antagonizing the facilitating effect of reserpine is likely to be of a different character from that of the agents just discussed. The interaction of lysergic acid diethylamide with 5-hydroxytryptamine is that of an antagonist, and this property is unlikely to play a part in its effect upon leptazol facilitation, since the similarly active compound 2-bromo-(+)-lysergic acid diethylamide was without effect. It is more reasonable to suppose that a central stimulant action of lysergic acid diethylamide is involved, since 2-bromo-(+)-lysergic acid diethylamide lacks such action, but it is, apparently, not an activity which has any effect upon the threshold of normal mice to leptazol.

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