Br. J. Pharmac. (1968), **34**, 27–31.

The effects of intracerebroventricularly administered noradnamine and other sympathomimetic amines upon leptazol convulsions in mice

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1. The influence of some intracerebroventricularly administered sympathomimetic amines on leptazol-induced convulsions has been investigated.

2. Noradnamine and high doses of dopamine proved to be anticonvulsant and also antagonized the facilitative effects of reserpine.

3. Noradrenaline and octopamine also antagonized reserpine-induced facilitation but were without effect on leptazol alone ; by contrast tyramine and small doses of dopamine lowered the threshold for leptazol convulsions.

4. A possible interpretation of these results is presented.

It has been suggested that dibenzocycloheptatrienes, structurally related to the tricyclic antidepressants (for example, imipramine, amitriptyline) might be formed *in vivo* from deaminated metabolites of the naturally occurring catecholamines and might be involved in the production of endogenous depressive illnesses (Roberts & Broadley, 1965). We have been interested, therefore, in comparing the actions of noradnamine (5-aminomethyl-2,3,7,8-tetrahydroxydibenzo [a,e] cycloheptatriene) with those of reserpine (often used to produce experimental depression in animals) on the central nervous system.

Preliminary studies have already shown that noradnamine administered intracerebroventricularly to mice produces sedation, hypothermia and akinesia without affecting the duration of barbiturate-induced sleep (Chambers, Redfern & Roberts, 1967). In reserpinized mice, however, which are also sedated, hypothermic and akinetic, barbiturate sleeping times are potentiated (Brodie, Shore, Silver & Pulver, 1955). Of the many tests available for comparing the effects of these two drugs, modification of the effects of leptazol-induced convulsions appeared to be ideal, because reserpine-like compounds, in contrast to other sedative agents such as barbiturates (Chen & Ensor, 1950), and chlorpromazine (Balestrieri, 1955), facilitate the effects of leptazol (Chen, Ensor & Bohner, 1954 ; Lessin & Parkes, 1959).

Methods

At least four doses of leptazol, dissolved in normal saline, were administered intraperitoneally to groups of twenty-five male albino Swiss mice (18–24 g), and the dose required to cause tonic extensor seizures within 30 min in 50% of the

animals (*ED*50) was determined graphically. Ninety-five per cent confidence limits were estimated by the method of Litchfield & Wilcoxon (1949). In other experiments reserpine (4 mg/kg) dissolved in 5% w/v ascorbic acid was injected intraperitoneally 3 hr before the leptazol (Chen & Bohner, 1961), and the *ED*50 was re-determined. The influence of noradnamine, noradrenaline, dopamine, tyramine and octopamine on each of these *ED*50 values was determined by injecting the amines (dissolved in saline containing 0.005% ascorbic acid) into the cerebral ventricles 15 min before the injections of leptazol. The technique used for the intercerebroventricular injections was similar to that of Haley & McCormick (1957), and has been described in detail elsewhere (Brittain & Handley, 1967). The injections were made through a 26-gauge needle $\frac{1}{8}$ in long, and our checks on the site of injection, using Indian ink, confirmed the findings of these other workers.

Drugs

(−)-Noradrenaline acid tartrate, dopamine hydrochloride, tyramine hydrochloride, (±)-octopamine hydrochloride (Koch-Light Laboratories Ltd.), reserpine (Ciba Laboratories Ltd.), and leptazol (Martindale Samoore Ltd.) were all obtained commercially. Noradnamine hydrochloride was synthesized in our own laboratories, by a process essentially similar to that for adnamine hydrochloride (Roberts & Broadley, 1967), although the refluxing of the reaction mixture was reduced from 12 hr to 20 min. Recrystallization was from distilled water. All doses in the text are expressed in terms of the appropriate base.

Results

All the data obtained are summarized in Table 1. In confirmation of the results of previous workers, reserpine facilitated the convulsive effects of leptazol, reducing

TABLE 1. *The influence of intracerebroventricularly administered amines on the leptazol ED*50 *in untreated and reserpine-treated mice*

Intracerebral amine (μg/20 g)	Untreated ED50 (mg/kg)	*P*†	Reserpine-treated ED50 (mg/kg)	*P*†
Saline control (20 μl./20 g)	77·3 (79·1–75·6)		52·6 (54·4–50·8)	
Noradnamine (10)	88·6 (90·6–86·6)	<0·001		
Noradnamine (20)	106·9 (120·8–94·6)	<0·001		
Noradnamine (40)	238·0 (302·3–187·4)	<0·001	76·6 (81·5–72·0)	<0·001
(−)Noradrenaline (1)	78·7 (80·7–76·8)			
(−)Noradrenaline (5)	78·2 (81·8–74·8)		78·9 (84·3–73·8)	<0·001
(−)Noradrenaline (20)	79·6 (82·6–76·7)			
Dopamine (0·5)	72·9 (75·6–70·3)	0·02–0·01		
Dopamine (1)	71·1 (73·4–68·8)	<0·001	54·7 (57·3–52·2)	
Dopamine (5)	73·9 (77·1–70·8)		57·7 (61·0–54·6)	0·01–0·001
Dopamine (20)	73·8 (76·7–71·0)		56·7 (58·6–54·8)	0·01–0·001
Dopamine (50)	82·9 (86·7–79·2)	0·02–0·01	62·3 (65·5–59·3)	<0·001
Dopamine (100)	82·3 (86·2–78·5)	0·02–0·01	65·6 (70·8–60·7)	<0·001
Tyramine (20)	74·4 (77·4–71·5)			
Tyramine (50)	68·2 (71·1–65·4)	<0·001		
Tyramine (100)	66·6 (70·7–62·8)	<0·001	55·7 (60·7–51·1)	
(±)Octopamine (50)	78·7 (82·8–74·8)		55·4 (59·0–52·0)	
(±)Octopamine (200)	78·2 (81·4–75·1)		58·5 (60·8–56·2)	<0·001

Reserpine (4 mg/kg) was administered intraperitoneally 3 hr before leptazol.
The figures for the saline controls represent the pooled results from a series of experiments conducted at regular intervals throughout the investigation.
* Figures in parentheses indicate 95% confidence limits.
† *P* values for differences between saline control and intracerebral amines.

the *ED*50 from 77.3 mg/kg to 52.6 mg/kg. By contrast, noradnamine proved to be an anticonvulsant, its effectiveness in this respect increasing with increase in dose. The highest dose tested caused a three-fold increase in the *ED*50 of leptazol and completely antagonized the facilitative effect of reserpine. Noradrenaline also completely antagonized the reduction in the *ED*50 of leptazol produced by reserpine, but was without effect on leptazol alone. A similar situation was shown to exist using octopamine, although the reversal of reserpine facilitation obtained was far from complete. Tyramine, on the other hand, exhibited weak facilitative effects on leptazol alone but failed to influence the *ED*50 when already reduced by reserpine pretreatment. Small doses of dopamine (0.5–1.0 μg/20 g) also caused a lowering of the convulsion threshold to leptazol alone and were without effect on the facilitative action of reserpine. Larger doses (5–100 μg/20 g) produced qualitatively different effects and some degree of anticonvulsant activity against leptazol in both untreated and reserpine-treated animals was obtained.

As a general observation all the amines injected intracerebroventricularly produced sedation, but in addition it was noticed that animals treated with noradnamine alone developed symptoms resembling, but not identical to, clonic convulsions after 2 hr. This interesting phenomenon is being investigated further, but for the purposes of the present study it is necessary to state that in animals showing these "convulsions," leptazol, even in doses 10 times higher than the *ED*50, failed to elicit tonic extensor seizures.

Discussion

The present study has demonstrated an interesting difference between the two sedative drugs reserpine, which lowers the threshold for leptazol-induced convulsions, and noradnamine which raises it. As the convulsion facilitative effect of reserpine is completely antagonized by noradnamine, however, they may be acting by related mechanisms. The other amines discussed were administered in an attempt to establish these mechanisms.

Reserpine is known to deplete the brain of monoamines (Paasonen & Vogt, 1956; Holzbauer & Vogt, 1956; Pletscher, Shore & Brodie, 1956; Shore, 1962), and the observations that inhibitors of monoamine oxidase depress its facilitative action on leptazol (Chen & Bohner, 1961; Pfeifer & Galambos, 1967), and are themselves anticonvulsant (Prockop, Shore & Brodie, 1959), has led to the belief that the lowering of the convulsion threshold induced by reserpine results from a decreased brain monoamine concentration. The nature of the monoamine involved, however, is the subject of some controversy, because 5-hydroxytryptamine (Kobinger, 1958; Lessin & Parkes, 1959), dopamine (Schaepdryver, Piette & Delaunois, 1962), and noradrenaline (Pfeifer & Galambos, 1967) have all been implicated. The results of our present experiments with catecholamines favour noradrenaline because it is both qualitatively and quantitatively superior to dopamine in antagonizing the facilitative effect of reserpine; the observation that noradrenaline is without effect on the convulsion threshold of animals given leptazol is therefore an anomaly, although this unexpected situation has been described before without explanation (Schmidt & Matthies, 1962).

If the facilitative effect of reserpine is the result of a lowered brain noradrenaline concentration, however, it follows that the noradrenaline present in the brain before

reserpinization should have been having an inhibitory effect. Certainly an inhibitory effect of noradrenaline has often been demonstrated at ganglionic synapses (Norberg & Sjöqvist, 1966), and on neurones in many parts of the central nervous system (Salmoiraghi, 1966), but the mechanism involved and the site(s) of action are far from clear. Noradrenaline could, for example, be acting as an inhibitory synaptic transmitter in its own right, or as an antagonist to the release of (presynaptically), or the action of (post-synaptically), an excitor transmitter. Its inhibitory effect on neuronal transmission might also, of course, be resulting from an action on the post-synaptic membrane by way of synapses related to, but not synonymous with, those involving the excitor transmitter, as has already been envisaged both for ganglia (Eccles & Libet, 1961), and for the central nervous system (Malcolm, Saraiva & Spear, 1967).

In explanation of our observed anomaly, therefore, we suggest that perhaps noradrenaline injected into the cerebral ventricles might readily refill the stores depleted by reserpine to re-establish the inhibitory function and hence antagonize the reserpine-induced facilitation, but cannot directly reach the site(s) at which endogenous noradrenaline normally acts to inhibit central nervous transmission.

This concept can also be used to explain the effects of dopamine if the lowering of the *ED*50 of leptazol produced by low doses is considered to be a direct action and the anticonvulsant effect of larger doses the result of the conversion of dopamine to noradrenaline in endogenous stores. If this were so, then in the experiments where the *ED*50 of leptazol has already been markedly reduced by reserpine, small doses of dopamine might be expected to be without effect, whereas the transmission inhibitory sites postulated would be accessible to the excess of endogenous noradrenaline resulting from the larger doses of dopamine.

Some support for the suggestion that noradrenaline and dopamine have opposite actions is derived from the results obtained with octopamine and tyramine. The former was weakly noradrenaline-like (no effect on leptazol alone but antagonism of reserpine-induced facilitation), and the latter was weakly dopamine-like (decrease in the leptazol *ED*50); octopamine, like noradrenaline, as a hydroxyl group on the β-carbon atom, whereas tyramine, like dopamine, has not. These observations may, of course, be coincidental, but the absence of noradrenaline-like effects following the administration of tyramine does suggest that it is not producing its effects in the central nervous system by causing the release of endogenous noradrenaline; the fact that tyramine (unlike reserpine) does not deplete noradrenaline from sympathetic ganglia (Fischer & Snyder, 1965), may be related to this observation.

It therefore seems unlikely that the anticonvulsant activity of noradnamine is associated with its recently demonstrated indirect sympathomimetic action (Broadley & Roberts, 1967); in the absence of additional knowledge concerning the pharmacology of noradnamine, further discussion at this stage would be unwise.

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(*Received December* 28, 1967)