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INTRAVENTRICULAR ADMINISTRATION OF RESERPINE AND ITS METABOLITES TO CONSCIOUS CATS

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Intraventricular injection of reserpine in conscious cats produced relaxation of the nictitating membrane, miosis with narrowing of the palpebral fissure to a slit, avoidance of light, diarrhoea, anorexia, and tranquillization. Equivalent doses of trimethoxybenzoic acid, reserpic acid or methylreserpate separately or in combination did not produce such effects, indicating that these reserpine metabolites were not involved in these reserpine actions. 5-Hydroxytryptamine alone or in combination with subthreshold doses of reserpine also did not duplicate the effects observed after effective doses of reserpine. The possible central sites of reserpine action are discussed.

In a recent review Bein (1956) suggested that reserpine may not act *per se*, but may be converted into an active substance in the tissues, or release an active substance (5-hydroxytryptamine), or act only in concert with the latter. The first suggestion was supported by studies on the metabolism of ^{14}C-labelled reserpine which indicated that very little reserpine was localized in the brain whereas much higher concentrations of its metabolites were present (Numeroff, Gordon, and Kelly, 1955 ; Sheppard, Lucas, and Tsien, 1955 ; Glazko, Dill, Wolf, and Kazenko, 1956). Further support was obtained when Gaddum and Vogt (1956) showed that 10 μg. of reserpine intraventricularly had no effect, and Sturtevant and Drill (1956) reported that 100 μg. intraventricularly caused an autonomic nervous system discharge followed by a typical reserpine tranquillization 24 hr. later. However, these doses may have been either too small or too large to induce typical reserpine effects within a time interval approaching that observed following other routes of administration. Furthermore, Peets and Schulert (1957) recently found that totally labelled ^{14}C-reserpine reached its highest brain concentration within 20 min. Plummer, Sheppard, and Schulert (1957), using tritium-labelled reserpine, showed a significant concentration of the drug in the brain for periods up to 48 hr. Plummer *et al.* (1957), therefore, suggested that reserpine might act *per se* and not through an indirect mechanism. In an attempt to elucidate the problem, we have studied the effects produced in conscious cats by the intraventricular injection of reserpine and its metabolites, as well as 5-hydroxytryptamine.

METHODS

Five cats had cannulae implanted in the right lateral ventricle according to Feldberg and Sherwood (1953). Studies were made on the behavioural and other changes produced when the drugs were administered in saline solutions in a total volume of 0.2 ml. When required, the *p*H of the solutions was adjusted to 7.0. All doses of the following drugs refer to the free acid or base: reserpine phosphate, trimethoxybenzoic acid, reserpic acid, methylreserpate, and 5-hydroxytryptamine creatinine sulphate (5-HT). All drugs except the last were studied by the blind technique and another independent observer always checked the observations of the authors. Observations covered a period of 6 to 8 hr. after injection, and drugs were given only once a week.

RESULTS

Reserpine.—The intraventricular injection of 10 to 16 μg. of reserpine produced no detectable behavioural or other effects in the cats. This confirmed the observation of Gaddum and Vogt (1956). After a latent period of 30 min., 18 to 20 μg. of reserpine caused relaxation of the nictitating membrane, miosis with narrowing of the palpebral fissure to a slit, squinting in response to light, withdrawal to the darkest corner of the cage, diarrhoea, anorexia, tranquillization, and a pronounced generalized depression. The anorexia was still present the following day, but the other effects appeared to be terminated in 24 hr. When

the dose of reserpine was increased to 40 μg., the same responses were obtained, but the latent period preceding their onset was reduced to 20 min. With both doses, normally aggressive cats became docile and fearful cats became friendly. In one experiment in which there was a high noise level, 40 μg. of reserpine produced no tranquillization and the cats remained alert. These observations agree with those of Bein (1956) but disagree with the findings of Sturtevant and Drill (1956). The differences are probably related to the 100 μg. dose of reserpine used by the latter investigators.

Reserpine Metabolites.—Intraventricular injection of 20 μg. of trimethoxybenzoic acid caused no reaction. Increasing the dose to 40 μg. resulted in decreased alertness and a slight anorexia. This latter effect differed from that produced by reserpine, because the animals would eat if encouraged to do so.

Intraventricular injection of 20 μg. of reserpic acid produced only defaecation and the animals appeared normal and consumed their usual quantity of food. Increasing the dose of reserpic acid to 40 μg. caused a decreased alertness but no tranquillization. One cat developed convulsive seizures affecting the left side 57 min. after injection. These seizures were of 30 sec. duration in the beginning but became continuous after 1.5 hr. They could be induced by loud noises. During the seizures the cat had a vacant stare, mydriasis, piloerection and howled continuously. Intraperitoneal injection of 30 mg./kg. of sodium pentobarbitone anaesthetized the cat and terminated the convulsions.

Intraventricular administration of 20 to 40 μg. of methylreserpate produced no effects in the cats other than a slight decrease in alertness.

When mixtures containing 20 μg. and 40 μg. each of trimethoxybenzoic acid, reserpic acid and methylreserpate were given, there were no significant changes in the cats.

5-Hydroxytryptamine.—Intraventricular injection of 100 μg. of 5-HT produced effects similar to those described by Feldberg and Sherwood (1954) and Gaddum and Vogt (1956). Increasing the dose to 500 μg. increased the degree of sedation, but its duration was only 1 hr. There was no anorexia and the overall effects of 5-HT were not the same as those seen after reserpine. Gaddum and Vogt (1956) have reviewed the evidence concerning the actions of reserpine and their relationship to its known ability to release 5-HT in the brain. They pointed out both the similarities and dissimilarities between the two drugs and observed an increase in lethargy when 5-HT was given intraventricularly 16 hr. after reserpine. If the action of reserpine is based upon 5-HT release, it might be possible to duplicate the effects of the former by simultaneous intraventricular administration of subthreshold doses of both drugs. Administration of 10 μg. of reserpine and 100 μg. of 5-HT was entirely ineffective in duplicating the effects seen when either reserpine or 5-HT were given alone. However, one cat died of respiratory paralysis 4 hr. after receiving the mixture. Tonic seizures preceded death, but no definitive cause of death could be established at necropsy. Furthermore, such reactions were not observed in the other animals.

Discussion

The results presented here indicate that typical central effects ascribed to reserpine are produced by minute quantities of this drug *per se*, and they cannot be duplicated by its metabolites either alone or in combination. The central actions of 5-HT, although similar in certain aspects, are not entirely the same as those of reserpine and do not have a comparable duration of action. Furthermore, a combination of subthreshhold doses of both drugs given simultaneously does not duplicate the effects seen after either drug alone. Thus our results would tend to answer the questions posed by Bein (1956) and indicate, in agreement with Plummer *et al.* (1957), that the central effects seen after administration of reserpine are related to that drug *per se*. The time interval for the onset of effect agrees with that found by Peets and Schulert (1957), and is probably related to the interval required for the drug to move throughout the ventricular spaces, diffuse into the tissues and attach itself to receptors. There can be little doubt that the effects observed have their origin in central sites because of the mode of administration and the small quantity of reserpine required for their production. However, it is possible that not all of the effects are a result of direct drug-receptor combinations, because activation of remote sites through interneuronal connexions may take place under the conditions of our experiments. On the other hand, certain definitive sites must be implicated in producing the effects observed. Stimulation of the parasympathetic sites in the anterior hypothalamus would account for the relaxation of the nictitating membrane and the miosis. The defaecation could result from stimulation of the pre- and supra-optic nuclear areas of the hypothalamus (Sheenan, 1940). The tranquillization and generalized depression probably involve the ascending

reticular formation with its collaterals in the hypothalamus because the area has been shown to be concerned with states of consciousness (Magoun, 1951–2). Moreover, our results support the suggestion of Schneider and Earl (1954) that changes have been produced in the central regulating mechanism of the autonomic nervous system in the brain stem.

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References

Bein, H. J. (1956). *Pharmacol. Rev.*, **8**, 435.
Feldberg, W., and Sherwood, S. L. (1953). *J. Physiol.*, **120**, 3P.
—— —— (1954). Ibid., **123**, 148.
Gaddum, J. H., and Vogt, M. (1956). *Brit. J. Pharmacol.*, **11**, 175.
Glazko, A. J., Dill, W. A., Wolf, L. M., and Kazenko, A. (1956). *J. Pharmacol.*, **118**, 377.
Magoun, H. C. (1951–52). *An Ascending Reticular System in the Brain Stem, The Harvey Lectures*, p. 53. New York: Academic Press, Inc.
Numeroff, P., Gordon, M., and Kelly, J. M. (1953). *J. Pharmacol.*, **115**, 427.
Peets, E. A., and Schulert, A. R. (1957). *Abst. of Papers, Div. of Biol. Chem., Amer. Chem. Soc., Miami, Florida, April* 7–12, p. 70C.
Plummer, A. J., Sheppard, H., and Schulert, A. R. (1957). *Abst. Int. Symposium on Psychotropic Drugs, Milan, Italy, May* 9–11, p. 50.
Schneider, J. A., and Earl, A. E. (1954). *Neurology*, **4**, 657.
Sheenan, D. (1940). *Res. Publ. Ass. Research Nervous Mental Disease*, **20**, 589.
Sheppard, H., Lucas, R. C., and Tsien, W. H. (1955). *Arch. int. Pharmacodyn.*, **103**, 256.
Sturtevant, F. M., and Drill, V. A. (1956). *Anat. Rec.*, **125**, 607.