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EFFECTS OF INJECTION OF MEPESULPHATE INTO THE CEREBRAL VENTRICLES OF CATS

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The injection of the anticoagulant mepesulphate (Treburon) into the cerebral ventricles of cats resulted in tremor, salivation, tachypnoea, loud calling, and brief periods of various forms of seizures followed by progressive reduction in spontaneous movements and somnolence. The possible sites of action for these effects are discussed.

Recently it was shown (Joseph, Jindal & Patel, 1959) that the anticoagulant mepesulphate (Treburon), when injected intravenously into dogs, rabbits and pigeons, greatly reduced the duration of anaesthesia induced by intravenous pentobarbitone. As mepesulphate had no analeptic action in unanaesthetized animals, the effect in the anaesthetized animals was suggestive of a specific antagonism to barbiturates.

The distribution of barbiturates in the brain following their administration has been the subject of many investigations. There is no selective localization in the mesencephalic and diencephalic areas, but a greater sensitivity of these structures to barbiturates is often alleged. As these areas are readily reached through the intraventricular route, the effects of mepesulphate injected into the cerebral ventricles of cats were examined with a view to obtaining information about the site and mode of its action in the central nervous system. The results were also expected to give an indication as to further lines of study of its possible barbiturate antagonism.

METHODS

Under pentobarbitone sodium anaesthesia, a Collison cannula was implanted aseptically into the left lateral ventricle of cats, as described by Feldberg & Sherwood (1953). After recovery from the operation, the cats were used for intraventricular injection once a week.

Mepesulphate, a heparin substitute, which is the sodium salt of sulphated polygalacturonic acid methyl ester methyl glucosamide, was kindly supplied by Hoffmann-la-Roche & Co., Switzerland. It is readily soluble, giving a clear and stable solution. For injection into the cerebral ventricles, it was dissolved in 0.9% NaCl solution and the volume injected was always 0.2 ml.

RESULTS

An intraventricular injection in cats of 5 to 10 mg of mepesulphate resulted in tremor, salivation, tachypnoea, loud calling, and various forms of seizure, often

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associated with mydriasis, salivation and pilo-erection. There was further a progressive reduction in spontaneous movements with varying depths of somnolence. This was a late effect and persisted for many hours after the seizures had ceased.

Tremor was an early effect which began 1 to 4 min after the injection. It was fine in character, was present in the ears, neck, trunk, fore- and hind-quarters, and occurred in bouts lasting usually for about half an hour. Twitching and flickering of the ears were also observed.

Salivation was not observed in all cats. When it occurred, it began 10 to 15 min after the injection and continued for 30 to 60 min. The saliva was usually thin and not copious; occasionally it became thick and mucoid. During the seizures salivation was often pronounced.

Tachypnoea was a regular effect which began 7 to 8 min after the injection, increased during the following 20 min and persisted up to 3 hr. Respiration became shallow and its rate increased to between 80 and 100/min. In the first hour or two of this tachypnoea, there were frequent periods of extremely quick and shallow respiration when the rate rose to 200/min; in one animal panting developed.

Loud calling was a regular feature. Three to five minutes after the injection the cat began miaowing, which within a short time developed into loud calling. The calling occurred in bouts which at first were infrequent and lasted for short periods only, but later became more frequent and prolonged. During seizures the calling ceased, but it was resumed immediately afterwards and was then particularly loud, each call being drawn out. At the late stage of reduced motor activity and somnolence, the periods of calling became less frequent and shorter; the calling itself became plaintive and feeble.

Seizures began 10 to 30 min after the injection. The cat became suddenly motionless, had a vacant stare, looked bewildered and did not react to auditory or visual stimuli. There was bending of the head sideways and downwards often associated with rocking movements, abrupt ear movements, and circling towards the side. During the circling the cat did not place its forepaws correctly, but placed the back of its paws on the floor. There was also twitching of the eyelids, and jactitation of the muscles of mastication resulting in snapping of the lower jaw. The twitching of the eyelids and of the facial muscles was usually more pronounced on the side towards which the head was bent. Bending and circling were unrelated to the side of cannulation of the lateral ventricle. After the head was bent, the cat remained sitting motionless for several seconds with a vacant stare. These events occurred in frequently recurring bouts, each bout lasting rarely more than half a minute. In several cats these bouts were the precursors of clonic contractions. Other forms of seizure activity were general convulsions and blind charging. Sniffing, hissing and snarling were seen occasionally.

The clonic contractions started in the hind leg of the side towards which the head was bent, lasting for 10 to 25 sec. They were first of small amplitude and executed from extreme flexion of the leg whilst the contralateral hind leg was extended. They spread to the foreleg of the same side, and, as the legs of the other side remained extended, the cat turned on its side and sometimes rolled over. As the clonic

contractions of the hind leg continued, they became more frequent, their amplitude became larger and the flexion decreased. Before the contractions ceased their frequency decreased and the last few contractions were executed from the fully extended leg. Such brief periods of clonic contractions occurred at intervals of a few minutes and continued for 1 to 3 hr. General convulsions lasting for a few seconds sometimes followed the clonic contractions; at other times they occurred independently.

Blind charging was often preceded by a short period of extreme restlessness. Then the cat would suddenly charge ahead, running forward or springing upwards and sometimes clinging in an upright position to the side of the cage like a monkey. It would remain in this position for several seconds, staring vacantly. Such charging would be repeated 2 to 3 times in a single paroxysm.

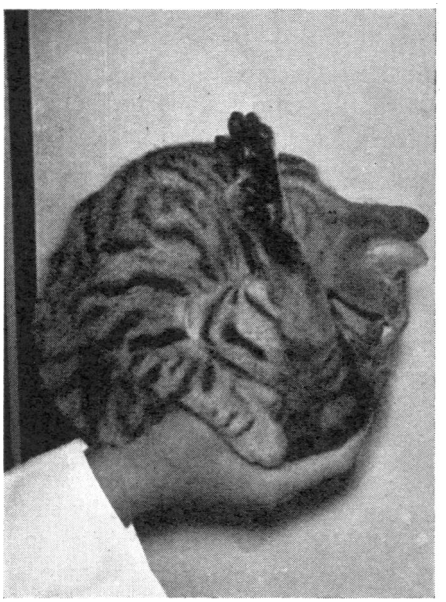

Fig. 1. Cat placed in the palm of the hand 3 hr after an intraventricular injection of 5 mg mepesulphate.

During the various forms of seizure activity, the pupils were dilated, but as the seizures became less vigorous, pupillary dilatation became less conspicuous. Intense pilo-erection on the root of the tail and on the back and flanks occurred often during the general convulsions and the blind charging.

Reduction in spontaneous activity with somnolence was seen in all cats. It began half an hour after the injection, increased progressively for 1 to 2 hr and then persisted for several hours after the seizures had ceased. The cat lay in the cage in a drowsy or sleep-like condition, opening its eyes or holding up its head only on strong stimuli. Some cats curled up as in natural sleep. When the condition deepened, as it did in several cats, flexion of the limbs, particularly of the hind limbs,

became a characteristic feature. When induced to walk the cat did not unflex its legs but progressed or, sometimes, retrogressed, on its belly. When held by the scruff of the neck, the head drooped and the limbs hung without struggling. The righting and placing reflexes were impaired and the cat could not land properly when dropped. If one foreleg were gently placed backwards across its body, it would be maintained in this position for over a minute without being withdrawn. Muscle tone was greatly reduced, and when the cat was held in a hand it assumed the position shown in Fig. 1 without struggling or resisting. There was, however, some muscle tone and the cat was able to perform movements such as scratching the ears with the hind leg. Twenty-four hours after the injection, no residual effects were present and the cats appeared normal.

With injections of 2.5 mg of mepesulphate, all effects were attenuated or absent.

DISCUSSION

Drugs injected into the lateral ventricles through an indwelling cannula pass quickly into the third and fourth ventricles and then into the subarachnoid space. The effects of mepesulphate, therefore, could result from action on structures reached from the ventricular cavities or from the subarachnoid space. However, the pattern of reactions observed when analysed in conjunction with the effects obtained by previous workers on stimulation of areas of the diencephalon and of the amygdala and the hippocampus suggests that intraventricular mepesulphate acts on one or more of these sites.

Many of the effects produced by intraventricular mepesulphate resemble those produced by certain other drugs, when injected intraventricularly. Notable among these is tubocurarine, which also produces tremor, loud calling and seizures, with pupillary dilatation, salivation and pilo-erection. The striking resemblance is suggestive of common sites of action.

Tremor-like movements are produced in monkeys by electrical stimulation of the medial regions of the brain stem from the mesencephalon to the medulla (Jenkner & Ward, 1953), but in cats the tremor-evoking areas extend further rostrally to the hypothalamus (Birzis & Hemingway, 1957). It is likely that substances evoking tremor on intraventricular injection act as chemical excitants of these areas. Domer & Feldberg (1960), investigating tremor following administration of drugs into the cerebral ventricles of cats, excluded the areas in the medulla and caudal portion of the mesencephalon as the main sites of action since tremor was obtained when the drugs were perfused from the lateral ventricles to the middle of the aqueduct. It appears likely that mepesulphate also evokes tremor mainly by an action on the mid-line structures in the diencephalon and mesencephalon. Since these areas are the sites of highest 5-hydroxytryptamine concentration in the brain (Amin, Crawford & Gaddum, 1954), and since 5-hydroxytryptamine itself elicits tremor on intraventricular injection, Domer & Feldberg suggested that this amine may be involved in the regulation of tremor activity. The question thus arises whether drugs like mepesulphate which produce tremor on intraventricular injection act on these regions directly or through the release of 5-hydroxytryptamine.

The various forms of seizure following intraventricular mepesulphate may well result from an action on the hippocampus and/or amygdala. In support of this assumption are recent results obtained with tubocurarine. Feldberg & Fleischhauer (1961) observed that the seizure discharge, produced by its intraventricular injection and associated with the convulsive activity, results from an action on structures reached by penetration from the posterior half of the lateral ventricle. They concluded that the structures concerned are the hippocampus and/or amygdala. These structures are characterized by a low seizure threshold for electrical stimulation (Green, 1960). Moreover, chemical excitation by injection of acetylcholine with physostigmine or of carbachol into the hippocampus also produces a seizure discharge (MacLean, 1957). Electrical stimulation of the amygdala (Gloor, 1960) elicits not only the seizure discharge but also the autonomic effects—mydriasis, salivation, pilo-erection and hyperpnoea—which are the accompaniments of the seizure activity produced by mepesulphate and tubocurarine. These effects obtained in response to intraventricular mepesulphate may therefore result from an action on the amygdala as well. Loud calling is a feature of the action not only of mepesulphate but also of tubocurarine when given by the intraventricular route. Could it be that this " symptom " is also the result of an action on the amygdala ? The occasional vocalization (Nacquet, 1953) and frequent growling (De Molina & Hunsperger, 1959) observed on electrical stimulation of the amygdala would be in favour of this possibility. On the other hand, Hess (1954) found that loud calling was a frequent response to electrical stimulation of areas in the wall of the third ventricle from the level of the subthalamus above to the infundibulum below, and extending to the grey stratum of the aqueduct. Thus the loud calling observed with mepesulphate can result as well from its penetration into diencephalic and mesencephalic structures. An action on these structures would also account for the late stage of diminished spontaneous activity and somnolence since it is known that these structures are involved in the sleep-walking phenomena.

Drugs which elicit convulsions usually counteract the depressant effects of barbiturates without being specific competitive antagonists, but exerting a functional type of antagonism. These considerations apply also to the barbiturate antagonism of mepesulphate since the present experiments have shown it to be a convulsant on intraventricular injection. On systemic administration, a convulsive effect is observed only when a very large dose is given. In mice 5.5 g/kg was required on intravenous injection to induce convulsions (Mangieri, Engelberg & Randall, 1951). If the mechanism of action underlying these convulsions is the same as that responsible for the convulsions obtained on intraventricular injection with a dose as small as 5 mg, this would suggest that the blood-brain barrier has a low permeability to mepesulphate.

Mepesulphate is an anticoagulant of heparin-like structure. It would therefore be interesting to know whether heparin and its other substitutes when given by the intraventricular route would produce a pattern of reactions similar to that of mepesulphate.

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