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INJECTIONS OF BULBOCAPNINE INTO THE CEREBRAL VENTRICLES OF CATS

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Recently it was found (Feldberg and Sherwood, 1954b) that the anticholinesterases dyflos (DFP) and eserine, when given to cats by the intraventricular route, produce a condition with many of the features of "catatonia." Experimental "catatonia" or "catalepsy" in animals is usually associated with the drug bulbocapnine and was first obtained with this substance by Peters (1904). There are numerous later publications (for reference see Ingram and Ranson, 1934; de Jong, 1945) on the action of bulbocapnine in producing this condition when given subcutaneously, but relatively large doses, between 20 and 30 mg./kg., are required to bring about the effect when administered in this way. In the present experiments it is shown that bulbocapnine is active in much smaller doses by the intraventricular route.

METHODS

The experiments were performed on nine cats with a permanent cannula in the lateral cerebral ventricle. The method of implanting the cannula and of injection was the same as that described previously (Feldberg and Sherwood, 1953; 1954a). The bulbocapnine used was the hydrochloride, a sample of which was kindly given us by Dr. H. Molitor, of the Merck Institute, Rahway, New Jersey. The salt was injected in Tyrode solution. As the solubility in this neutral solvent is relatively poor at room temperatures, it was necessary to dissolve the salt in heated Tyrode solution and to keep the fluid warm until the injection was made. In this way it was possible to inject the doses used in a small volume (0.3 ml.) without having to acidify the solution. The only effect sometimes obtained by control injections of 0.3 ml. of saline or Tyrode solution was a tendency to sleepiness or subduedness developing 1 to 1½ hr. after the injection.

RESULTS

An effect was obtained regularly with 1 mg., and set in after a latency of 30 sec. to 3 min. During the latency some of the cats became agitated and manifested difficulty in locomotion; the gait became unsteady and there was occasional staggering and stumbling; some cats retched and vomited; one cat defaecated and had erection of the penis. After these preliminary signs all movements became severely restricted and slow, and the cats showed signs of "catatonia" in response to the usual tests. The onset of this change in behaviour was often so sudden that it overtook the cat during the execution of a movement which it did not complete. For instance, a cat would stop with one paw off the ground, half-way through a step. At this stage the cat usually assumed a crouching position with its legs flexed well under its body. This is apparently the position described by Ingram and Ranson (1934), in their experiments with subcutaneous injections of bulbocapnine, as "flexion position," "flexion tendency" and "flexion attitude," by which they refer to "the disposition of an animal to assume a crouched position with the limb flexed beneath it and the back somewhat arched." There is, at the beginning of the "catatonic" phase, a period in which the respiration rate is raised up to 40/min.

The "catatonic" phase was recognized by the following phenomena. When the cat was put on the ground, it remained standing in the position in which it landed for some time. When induced to walk, it stopped after a few steps, standing motionless for long periods before it gradually lay down in a crouched position. When the hind quarters were lifted from the ground and released after a few seconds, the cat took two or three steps on its front paws with its hind quarters still pointing upwards and the back concave before lowering its hind quarters. When a front paw was abducted or placed across the cat's own spine, it remained in this position for periods varying between 30 and 45 sec. When put into the erect posture with its front paws resting on the upper rungs of an inverted stool, the cat remained in this position for about 20 sec. before slowly climbing down. Similarly, a cat placed with its groin across the lower rung of an inverted stool remained in this

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position for several seconds. The cat could be brought into a number of other unnatural attitudes by lifting its front or hind limbs, and it offered no resistance nor did it make any muscular effort to alter these positions. It had difficulty in extricating itself from awkward positions, but, when made to move or jump off a shelf, showed no signs of muscular incoordination.

The cat no longer reacted to events in the room, nor did it come forward when approached. A hand could be waved close to its face, eliciting nothing beyond a blinking of the eyes. It was also difficult to obtain the cat's attention by noises, but when stimulated by stroking, or inducing it to move, the cat betrayed its awareness by lashing its tail and changing its facial expression and, in several instances, by uttering hoarse cries which continued for some time after the stimulus ceased. Sometimes a cat uttered such hoarse cries of itself during the stage preceding "catatonia." The eyes were usually half shut, and an attempt to open the lids met with strong contraction of the orbicularis oculi muscles.

The "catatonic" phase was at its height 15–30 min. after the injection and gradually wore off during the following hour or two. This lightening of the animal's state was recognized by the fact that the cat could be made alert for short periods by persistent stimulation, that it was more easily induced to walk, or showed periods of spontaneous activity which were, however, still subject to sudden arrest and persistence in a pose.

With doses larger than 1 mg. there were additional features. In one cat, short-lasting general convulsions occurred during the first minute after an injection of 5 mg. bulbocapnine. Then followed a condition of deep "catatonia" lasting for over an hour. During this condition the cat, when hung on the upper rung of an inverted stool, supported itself with one forepaw only. Yet, when taken off the rung, it walked without signs of incoordination. Further, a hind leg twisted over its back returned only slowly to its normal position when the restraint was removed. When smoke was blown into the cat's face it made no withdrawal movements, but when touched it responded with howling or crying. When induced to step from one shelf to another, 3 in. higher, it ceased moving with one hind limb still remaining on the lower shelf, maintaining this position for minutes. At an early point in this "catatonic" state the cat, when undisturbed, lay flat on its belly and cried from time to time.

Another cat reacted to the intraventricular injection of 4 mg. bulbocapnine as follows. Within ½ min. it became unable to stand up. Although the legs were flexed, the claws were protruded and there was a pronounced grasp reflex, particularly on the forepaws. When a hind limb was touched there were contraction jerks of the limbs. In the course of about half an hour the cat regained the power to support itself; at first it walked clumsily, in an unsteady manner, and later on stiffly. When the usual tests for "catatonia" were applied it was found that the cat put up some perfunctory struggle before remaining in the abnormal position. Thus, when suspended with its forepaws on the upper rung of an inverted stool, it first struggled and began to move off but arrested this movement and remained fixed with one paw still holding on to the rung. It also struggled first when placed on both upper rungs of the inverted stool, but then remained there immobile until pushed off, when it jumped down and moved away without signs of incoordination. As with the other cat, under the effect of a large dose of bulbocapnine it showed some signs of awareness such as lashing its tail, changing its facial expression, and growling or purring according to the way it was handled.

Doses of bulbocapnine smaller than 1 mg. were too small to produce signs of "catatonia" consistently, but other striking changes in behaviour were observed.

One cat, for instance, during the first few minutes after an injection of 200 μg. of bulbocapnine had a laboured respiration; it vomited after some licking movements, salivated and retched. Ten to fifteen minutes later there was this pronounced change in the cat's previously docile and friendly behaviour: whenever approached it hit out with one or both front paws, sitting erect and hissing with bared teeth, without, however, following through with the attack. When left undisturbed, the cat paced about with quick and sudden movements, lashed its tail, and by its head movements in response to noises or tapping on the table betrayed heightened alertness. The animal was affected in this way for about 20 min. No signs of "catatonia" were produced by this dose of bulbocapnine. The same kinds of reactions —laboured respiration, vomiting, anger, but no feature of "catatonia"—were produced by the same dose on subsequent occasions.

In another cat the injection of 100 μg. immediately produced profound signs of "catatonia," as judged by the fact that the cat, or its limbs, could be placed without struggle in unnatural positions or attitudes which were retained for many seconds. In this state the cat often arrested the progress of

a movement and remained motionless in the posture thus acquired for minutes. When an attempt was made to dislodge a cat, on a broad shelf where it was being examined, by pushing against its shoulders or sides, it pressed in the opposite direction and anchored itself to the edge of the shelf with the paw of the appropriate side. The signs of "catatonia" were replaced after about 10 min. by behaviour characteristic of anger. Then, when an attempt was made to put its forepaw across its spine, the cat hit out and tried to bite, lashing its tail. This particular cat responded to a higher dose (200 μg.) with signs of intense itching and motor phenomena of convulsive quality. The cat started to scratch itself, to wipe and lick its paws with increasing vigour, until this activity became paroxysmal. Movements were ill controlled and jerky. The gait was unsteady and spastic, there was often typical scissor gait; the claws tended to close over any object and it was difficult to disengage them. The legs were extended and the basic posture was a crouching one with the body close to the ground; from time to time the cat rushed forward or backward, or circled in either direction, suddenly coming to a halt with eyelids and ears twitching. Within 20 min. the cat gained control over its movements and was then easily startled.

Discussion

Bulbocapnine, when injected intraventricularly, produces its characteristic effects in doses a fraction of those effective on subcutaneous injection. With both methods of application the effects are very similar, except that with intraventricular injection the effect sets in earlier and disappears more quickly than with subcutaneous injection. The time taken for absorption from the subcutaneous tissue explains this difference in the onset and duration of the effects.

With the present method of administration the relative smallness of the dosage and the speed with which the bulbocapnine effects appear indicate that structures close to or directly in contact with the ventricular surface are likely to be implicated. This indication is borne out by comparison with results obtained by lesions and stimulation of selected anatomical sites in the mid-brain close to the ventricular lining. From these, and only from these sites, effects analogous to those of bulbocapnine are obtained.

There are many observations (Ingram, Barris, and Ranson, 1936; Bailey, 1948; Magoun, 1952; MacCulloch, Ridley, and Sherwood, 1952; Hess, 1954) that lesions on the border between the upper tegmentum and the posterior hypothalamus produce a condition resembling the "catatonic stupor" of bulbocapnine. Moreover, not only "catatonic stupor" but also other signs such as those of anger and irritability, described here after bulbocapnine, have been elicited from these regions.

In his classical experiments with electrical stimulation of the diencephalon, Hess obtained hissing, snarling, excitement, and calling, apparently of a plaintive quality, with stimulation through electrodes in the following areas: the wall of the third ventricle from the level of the subthalamus above to the infundibulum below, including points close to the descending column of the fornix and the tracts of Vic d'Azur and an area extending caudad in the periaqueductal grey and the medial longitudinal bundle. Further, when lesions had been made by electro-coagulation in the area immediately above the mammillary bodies and rostral to the red nuclei, Hess obtained not only the typical signs of catatonic stupor, but some cats became hostile as the catatonic stupor wore off during subsequent weeks. A previously tame cat with a lesion in the posterior hypothalamus, bordering the tegmentum and involving the lining of the third ventricle as far caudad as the entry of the aqueduct, would hiss and hit out when touched or approached and then would move away in a crouching position without following up the attack. Another cat with a lesion only slightly further caudad and lateral to that in the previous cat, but this time not destroying the ependyma, uttered continuous plaintive cries when observed until it died three weeks later. Similar behaviour was seen by Sherwood, Ridley, and McCulloch (unpublished) in cats with lesions in the upper brain stem. The changes of behaviour in these cats recalls in a striking fashion those seen after intraventricular injections of bulbocapnine.

On intraventricular injection the anticholinesterases eserine and DFP regularly, before the onset of catatonic stupor, produced intense scratching, washing, and licking of the paws, giving the impression of itching (Feldberg and Sherwood, 1954b), an effect also obtained in one experiment with bulbocapnine. Hess found that cats acted thus on stimulation of the anterior intraventricular portion of the fornix, of the nuclei of the septum pellucidum as far ventrally as the anterior commissure, and of a region immediately above and behind the mammillary bodies—again structures bathed by the ventricular fluid. From some areas the effect was obtained during stimulation, from others immediately after it.

In animals, the main method of obtaining the syndrome of catatonia, apart from drugs, is the

placing of lesions in the upper brain stem. This raises the question whether bulbocapnine also produces a kind of pharmacological lesion by paralysing nerve cells and thereby interrupting some specific pathways, in analogy to the paralysing effects of nicotine or acetylcholine on peripheral structures when producing block of synaptic transmission in a sympathetic ganglion, or neuromuscular block. In this connexion it may be recalled that large doses of acetylcholine as well as of eserine and DFP will produce catatonia when injected intraventricularly.

The finding that in relatively large doses bulbocapnine, on intraventricular injection, evokes convulsions, as it does on subcutaneous injection, renders it likely that the convulsions are also of subcortical origin. The site of action may well be the thalamus, stimulation of which with high voltages is known to produce seizures of all kinds (Jasper and Droogleever-Fortuyn, 1947; Hunter and Jasper, 1949; Hunter, 1950; Kaelin, 1951). In its convulsive action on intraventricular administration, bulbocapnine has an effect in common with (+)-tubocurarine, acetylcholine, DFP, and eserine.

Summary

1. Intraventricular injections of 1 mg. bulbocapnine produce in cats a state of catatonic stupor; to produce this condition with subcutaneous injections doses at least forty times larger are required.

2. Intraventricular injections of 100–200 μg. of bulbocapnine which need not evoke catatonic stupor produce short-lasting changes in behaviour: a previously docile cat may show hostility.

3. The changes produced by bulbocapnine on intraventricular injections are correlated with similar changes obtained from electrical stimulation and from lesions at discrete points in the mid-brain.

The detailed description of the experiments of Professor W. R. Hess given in the Discussion was obtained during a visit by the authors for the purpose of working in his extensive "Brain Biological Collection" which is at present accommodated in the Anatomical Institute of the University of Zurich and consists of films, protocols, and histological preparations from his experiments. We should like to record our great indebtedness to Professor Hess for the hospitality and generous help we received.

References

Bailey, P. (1948). *J. nerv. ment. Dis.*, **107**, 336.
Feldberg, W., and Sherwood, S. L. (1953). *J. Physiol.*, **120**, 3*P*.
—— —— (1954a). Ibid., **123**, 148.
—— —— (1954b). Ibid., **125**, 488.
Hess, W. R. (1954). *Das Zwischenhirn.* Basle: Schwabe.
Hunter, J. (1950). *Electroenceph. clin. Neurophysiol.*, **2**, 193.
—— and Jasper, H. H. (1949). Ibid., **1**, 305.
Ingram, W. R., Barris, R. W., and Ranson, S. W. (1936). *Arch. Neurol. Psychiat., Chicago*, **35**, 1175.
—— and Ranson, S. W. (1934). Ibid., **31**, 987.
Jasper, H. H. D., and Droogleever-Fortuyn, J. (1947). *Res. Publ. Ass. nerv. ment. Dis.*, **26**, 272.
Jong, H. H. de (1945). *Experimental Catatonia.* Baltimore: Williams and Wilkins.
Kaelin, R. (1951). *Experimentell induzierte epileptiforme Syndrome.* M.D. thesis, University of Zurich.
MacCulloch, W. S., Ridley, E., and Sherwood, S. L. (1952). *Nature, Lond.*, **169**, 157.
Magoun, H. (1952). *Arch. Neurol. Psychiat., Chicago*, **67**, 145.
Peters, F. (1904). *Arch. exp. Path. Pharmak.*, **51**, 130.