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PHARMACOLOGICAL EFFECTS PRODUCED BY INTRACEREBRAL INJECTION OF DRUGS IN THE CONSCIOUS MOUSE

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A method has been described for the study of the central effects produced by the intracerebral injection of drugs in the unanaesthetized mouse. The effects observed were in good agreement with those obtained after similar injections in cats, dogs and human beings. After intracerebral injection, drugs of diverse structure produced certain generalized effects: changes in positioning of the tail, stupor, hyperexcitability and tachypnoea. Both acetylcholine and methacholine produced an akinetic seizure and depression, but the latter compound also caused lacrimation and salivation. Atropine produced piloerection, increased sensitivity to sound and touch, clonic convulsions and scratching, whereas hexamethonium caused Parkinsonian-like muscle tremors and peripheral vasodilatation. After adrenaline, hyperexcitability, exophthalmos, stupor and death from pulmonary oedema were observed, but (+)-methylamphetamine produced only piloerection and exaggerated activity in response to sound and touch. Ergotamine caused a decreased sensitivity to sound and touch, micturition, and stupor, while ergometrine caused clonic convulsions, piloerection, defaecation and stupor.

There is much current interest in the pharmacological effects observed after the administration of drugs into the brain of conscious animals. Such procedures give a better estimation of the central actions of drugs because diffusion through the blood-brain barrier is not involved. Furthermore, the doses required to produce an effect by this mode of administration are, in general, much less than those required by other routes. Feldberg and Sherwood (1953a and b, 1954a and b, 1955) have approached the problem by injecting various drugs into the lateral ventricles of cats by an implanted cannula. A similar technique has been used in dogs (Haley and Weinberg, 1955; Haley and Dickinson, 1956; Haley and McCormick, 1956). However, such animal preparations are too expensive for routine screening experiments, although they are useful for exploring sites of central activity as distinguished from peripheral ones. As a possible solution for the problem of screening for central activity, we have devised a simple method using direct intracerebral injection in the mouse.

METHODS AND MATERIALS

Two hundred and fifty mice (strain CF 1) of either sex, weighing 20 to 25 g. each, were used. Physiological saline solutions of the drugs studied were injected into groups of ten mice at each dose used. The animal was grasped firmly by the loose skin behind the head. The skin was pulled taut. A $\frac{1}{8}$ in., 27 gauge hypodermic needle attached to a 0.25 ml. syringe was inserted perpendicularly through the skull into the brain and 0.01 to 0.05 ml. of solution was injected. The site of injection was 2 mm. from either side of the midline on a line drawn through the anterior base of the ears (Fig. 1). For ascertaining the areas in the brain ventricular system into which the drugs penetrated, 0.05 ml. of a 1:10 dilution of Indian ink was injected, and the brains were sectioned and studied histologically. All drugs and control injections were tested in groups of ten animals.

RESULTS

The site of injection (Fig. 1) was critical because penetration into the ventricles was not accomplished by injecting further rostrally and too much damage to vital centres was produced by injecting more caudally. The size and length of the needle was also critical for the same reasons and, in addition, an increase in length resulted in extensive brain damage from movement during injection.

The distribution of the carbon particles in Indian ink injected into the ventricular system with this technique showed that drugs, given by

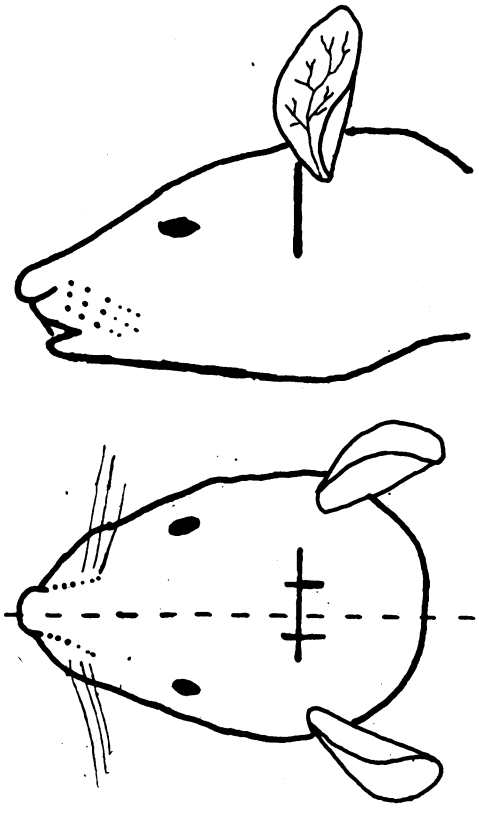

FIG. 1.—External landmarks for locating site of injection.

this route, could be expected to influence the activity of the vital centres located in the walls of the ventricles. Neuronal links between these and other centres in the brain might modify the responses observed. Passage of some of the injected material through the foramina of Luschka and Magendie might produce still other effects.

Insertion of the needle or injection of 0.01 to 0.05 ml. of physiological saline solution had a slight effect on the mice. Immediately after removal of the needle, the animals remained quiet for approximately one minute and then resumed their normal activity. None of the control animals showed any residual or detrimental effects from the procedure.

Acetylcholine.—Immediately after the intracerebral injection of 1 μg. of acetylcholine, the mice assumed a hunched-up position with their hind legs spread far apart. The animals appeared dazed and remained in this fixed position for two minutes. The position resembled an akinetic seizure. The Straube tail phenomenon was also observed at this time. The animals were highly excitable and would move rapidly if touched. If left alone, they remained in one position and appeared to be depressed. .This latter condition lasted 10 to 15 min., after which the animals resumed their normal pre-injection activity. When the dose was increased to 10 μg. the above effects were more pronounced and the depressed condition lasted 30 to 40 min. The mice showed no residual effects 24 hr. later.

Methacholine.—Immediately after administration of 1 μg. of methacholine, the respiration was slowed and lacrimation and salivation were observed. The tail relaxed and remained parallel to the body. A depression of 10 to 15 min. duration was seen. An akinetic seizure similar to that seen with acetylcholine also occurred. When the dose was increased to 10 μg., the same effects were produced, but the depression appeared to be greater though its duration was still only 15 to 20 min. After both doses, the mice resumed their normal activity when the depression passed.

Atropine.—Intracerebral injection of 10 μg. of atropine caused increased respiration, a relaxation of the tail and a flattening of the ears against the head. Although the mice appeared depressed, they were extremely sensitive to both sound and touch. A weak stream of air would cause the animals to jump vertically several inches. Immediately after the injection of 100 μg. of atropine the mice had tachypnoea and piloerection. This was followed by bradypnoea and a generalized depression of 3 to 5 min. duration. During the next 15 to 30 min. the animals had clonic seizures and were extremely sensitive to sound and touch. A generalized itching sensation was also present because the animals scratched their bodies and particularly their noses. Fatigue and depression, lasting 2 to 3 hr., followed. Increasing the dose of atropine to 200 μg. decreased the primary depression to 2 min. and increased the period of clonic seizures to 30 to 45 min. and the secondary depression to 3 to 4 hr. All animals appeared normal 24 hr. later.

Hexamethonium Chloride.—A 1 μg. dose of hexamethonium produced tachypnoea, a relaxation of the tail and a drawing back of the ears. After a 15 to 30 min. period of depression, the mice resumed their pre-injection activity. Similar effects were observed after a 10 μg. dose, but the depression lasted 3 to 4 hr. During this period, the mice responded to sound or touch with generalized muscle tremors resembling those seen in a Parkinsonian syndrome. In addition, there was

a marked dilatation of the blood vessels of the legs and tail, and the latter was hot to the touch in contrast to the cold tails of the uninjected control animals. Increasing the dose to 50 μg. resulted in the same response seen after the 10 μg. dose. None of the animals died or showed any evidence of residual effects 24 hr. later.

Adrenaline.—Injection of 1 μg. of adrenaline caused an immediate increase in respiration, hyperexcitability and exophthalmos. The tail was elevated perpendicular to the body. After 15 min. the mice became sedated and could not be aroused. Normal activity partially returned in 30 to 40 min., but complete recovery required 5 to 8 hr. A 10 μg. dose produced an increase in respiration and a Straube tail phenomenon, followed shortly by stupor and death from pulmonary oedema. During the development of the oedema, the respiratory movements became weaker and gradually ceased.

(+)-*Methylamphetamine.*—Intracerebral injection of 100 to 200 μg. produced a rapid respiration, piloerection, a Straube tail phenomenon and exaggerated activity in response to sound and touch. All of these effects lasted from 1 to 4 hr. No residual effects were present 24 hr. later.

Ergotamine.—A 1 μg. dose of ergotamine caused tachypnoea, a flattening of the ears against the skull and a Straube tail phenomenon. Later the tail became straightened out behind the animal. A slight stupor with decreased response to touch and sound was also seen. Normal activity resumed in 10 to 15 min. Injection of 5 μg. produced tachypnoea followed by bradypnoea, urination, ears flattened against the head and a relaxed straight tail. The stuporous state was more pronounced, but the animal resisted attempts to change its position. There was a slight response to touch but not to sound. Normal activity returned in 30 to 40 min. and there was no detectable residual changes in the animals 24 hr. later.

Ergometrine.—An injection of 1 μg. of ergometrine produced the following effects: tachypnoea followed by bradypnoea, ears flattened against the head, a relaxed tail and stupor. The animals responded to sound with a clonic seizure. 20 to 45 min. later, normal activity was resumed. The same effects were produced by a dose of 5 μg., but, in addition, piloerection, defaecation and a Straube tail phenomenon were observed. There was only a slight response to stimulation, but the animals showed normal responsiveness 15 to 20 min. later. They appeared normal the next day.

Discussion

Depression similar to that described here in mice has been reported previously in cats (Dikshit, 1935; Silver and Morton, 1936; Bornstein, 1946; McCulloch, Ridley, and Sherwood, 1952; Feldberg and Sherwood, 1954a) and in man (Henderson and Wilson, 1936) receiving intracerebral injections of acetylcholine. The akinetic state was observed in cats by Feldberg and Sherwood (1954a).

The effects of atropine in the mouse were similar to those reported in the cat by Feldberg and Sherwood (1954a). Although no fatalities occurred, the higher doses presented a typical picture of the central effects usually observed after toxic doses of atropine.

The effects observed after intracerebral administration of hexamethonium in mice were in many ways similar to the responses obtained by Feldberg and Sherwood (1954a) in the cat. However, the peripheral vasodilatation was not observed in cats, and this is difficult to explain in terms of a block of transmission in peripheral ganglia. On the other hand, vasodilatation could be caused by a direct effect on the vasomotor centre.

A condition resembling sleep was the characteristic effect produced by the intracerebral administration of adrenaline in mice. A similar effect has been seen in dogs (Bass, 1914; Leimdorfer and Metzner, 1949), in cats (Feldberg and Sherwood, 1954a) and in human beings (Leimdorfer, Arana, and Hack, 1947; Leimdorfer, 1948). The fatal pulmonary oedema following central administration of adrenaline appeared to be characteristic of the rodent and has been reported earlier by Cassen and Kistler (1954).

Hyperexcitability also appeared to be a characteristic response to sympathomimetic amines because both adrenaline and methylamphetamine produced this effect. The longer duration of action of the latter compound following intracerebral injection was in accord with the results obtained by other routes of administration (Haley, 1947).

The decreased motor activity of the mice after ergotamine was similar to that reported in cats after intraventricular injection of ergotamine (Schwartz, Wakin, Bickford, and Lichtenheld, 1956). A stuporous state seemed to be the characteristic response produced in mice after intracerebral injection of ergot alkaloids. A similar response has been seen after the administration of these drugs into the third ventricle in cats (Goodman and Gilman, 1955). The clonic convulsions produced by ergometrine appeared to

be a characteristic action of this alkaloid because ergotamine did not produce this effect in the same dose.

Screening techniques require the establishment of the validity of the procedure in relation to what is already known concerning the particular drug(s) and function(s) being studied. Difficulties are attached to the study of centrally induced peripheral effects where the species under consideration is of the rodent family, since certain functions or reflexes are absent, such as the emetic reflex. However, valid results can be obtained if such differences in neurological development are borne in mind. There can be little doubt, from the data presented, that the intracerebral injection technique in the mouse can be used to study centrally induced peripheral effects. The mouse responses to drugs were, in the main, similar to those in other species, including human beings. The rapidity of response after intracerebral injection precluded any direct action on the peripheral receptors. However, there was the possibility, as pointed out earlier by Bedford (1953), that leakage of the drug through the needle track followed by absorption from the subarachnoid space into the systemic circulation could take place. There was also the fact that the volume injected, 0.01 to 0.05 ml., was greater than the volume of the cerebrospinal fluid. Thus, if the drug leaked out of the foramina and passed over the external surface of the brain, it might activate many other sites of nervous activity by a direct action. Under these circumstances absorption into the systemic circulation could take place. However, the amount of drug involved would seem to mitigate against such a possibility, particularly when one considers the dosage differential (20 to 40 times) required to produce pharmacological effects after parenteral administration.

Aside from its value as a screening procedure, the method could be used for student demonstration of the direct effects of drugs on the central nervous system in the absence of direct peripheral effects. It could also illustrate the possibility of centrally induced side effects. However, as many drugs do not pass the blood-brain barrier, the demonstration of centrally induced effects under such circumstances may be only of academic interest.

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References

Bass, A. (1914). *Z. ges. Neurol. Psychiat.*, **26**, 600.
Bedford, T. H. B. (1953). *J. Physiol.*, **120**, 62P.
Bornstein, M. B. (1946). *J. Neurophysiol.*, **9**, 349.
Cassen, B., and Kistler, K. (1954). *Amer. J. Physiol.*, **178**, 53.
Dikshit, B. B. (1935). *J. Physiol.*, **83**, 42P.
Feldberg, W., and Sherwood, S. L. (1953a). Ibid., **120**, 3P.
—— —— (1953b). Ibid., **120**, 12P.
—— —— (1954a). Ibid., **123**, 148.
—— —— (1954b). Ibid., **125**, 488.
—— —— (1955). *Brit. J. Pharmacol.*, **10**, 371.
Goodman, L. S., and Gilman, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 581. New York: Macmillan.
Haley, T. J. (1947). *J. Amer. Pharm. Assoc., Sci. Ed.*, **36**, 361.
—— and Dickinson, R. W. (1956). Ibid., **45**, 432.
—— and McCormick, W. G. (1956). *Fed. Proc.*, **15**, 433.
—— and Weinberg, S. J. (1955). *Proc. Soc. exp. Biol., N.Y.*, **89**, 345.
Henderson, W. R., and Wilson, W. C. (1936). *Quart. J. exp. Physiol.*, **26**, 83.
Leimdorfer, A. (1948). *Wien. klin. Wschr.*, **60**, 382.
—— Arana, R., and Hack, M. A. (1947). *Amer. J. Physiol.*, **150**, 588.
—— and Metzner, W. R. T. (1949). Ibid., **157**, 116.
McCulloch, W. S., Ridley, E., and Sherwood, S. L. (1952). *Nature, London*, **169**, 157.
Schwartz, B. E., Wakin, K. G., Bickford, R. G., and Lichtenheld, F. R. (1956). *Arch. Neurol. Psychiat. Chicago*, **75**, 83.
Silver, G. A., and Morton, H. G. (1936). *J. Pharmacol.*, **56**, 446.
Weinberg, S. J., and Haley, T. J. (1955). *Circulation Research*, **3**, 103.
—— —— (1956). *Arch. int. Pharmacodyn.*, **105**, 209.