A STUDY OF THE PENETRATION OF CERTAIN CHOLINOLYTIC DRUGS THROUGH THE BLOOD-BRAIN BARRIER BY PERFUSING THE CEREBRAL VENTRICLES

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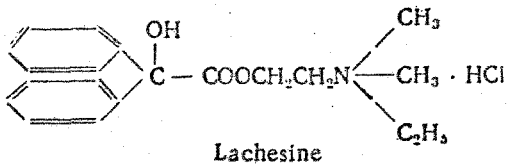
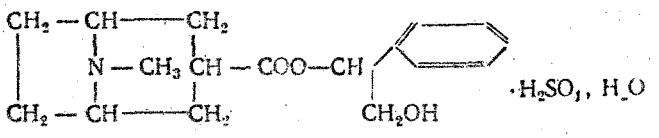
When the central action of cholinolytic substances is being assessed, differences in effect are sometimes attributed to variation in the ability of these drugs to pass through the blood-brain barrier (BBB). For example, the absence of any marked central cholinolytic action of many compounds containing a quaternary ammonium group is associated with the fact that they pass through the BBB with difficulty. In contrast to these, many compounds whose molecule contains a tertiary nitrogen atom passess a well-marked central cholinolytic action. It is therefore assumed that all tertiary cholinolytics pass readily through the BBB, while the differences in their central cholinolytic effects are ascribed to actual differences in cholinolytic activity.

Interesting results have been obtained by comparing the central effects of cholinolytics when injected directly into the brain and into the general circulation [1, 6], and also into the blood vessels supplying mainly certain divisions of the brain [8]. It has been found that tertiary and quaternary cholinolytics, if injected into the cerebral ventricles, give the same cholinolytic effect, although the cholinolytic activity of the latter far exceeds that of the former. If cholinolytics are injected into the blood stream, the tertiary compounds have a marked central action while the quaternary give no such effect. No differences have been found as regards the passage of individual tertiary cholinolytics through the BBB.

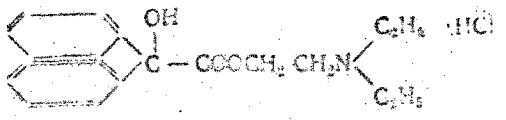
In the present investigation tests were made of the ability of three cholinolytics to pass through the BBB: one quaternary – lachesine (β-benziloyloxyethyldimethylethylammonium chloride [13]) and two tertiary – atropine (sulfate of the tropine ester of tropic acid) and amizil (hydrochloride of the 2-diethylaminoethyl ester of benzilic acid), with the following structural formulas:



Lachesine



Atropine



Amizil

EXPERIMENTAL METHOD

The method of perfusion of the cerebral ventricles, suggested by Adam and co-workers [11], was used to assess the passage of the cholinolytics through the BBB. A. P. Golovin [4] used this method in 1948 to study the effect of drugs on the dog's respiratory center. In our experiments we used the technique of perfusion of the cerebral ventricles suggested by Bhattacharya and Feldberg [12] for cats.

The lateral and third ventricles, the aqueduct, the fourth ventricle, part of the subarachnoid space, and the suboccipital cistern. Ringer-Locke's solution was first heated to 39° and aerated. Perfusion was carried out at the rate of 7-8 drops per minute. It was important in these experiments to maintain an optimal temperature of the solution (39°) and to perfuse at a constant rate. For this purpose we adapted a type TS 15 M ultrathermostat.

The investigation was conducted on 27 cats. The cholinolytic drugs were injected into the carotid artery in a dose of 4 mg/kg body weight. This dose did not produce toxic effects in the experimental animals. The perfusion fluid was collected from the suboccipital space every 30 min and its cholinolytic activity was immediately tested on the isolated rat's ileum. Usually the first sample of perfusion fluid before injection of the cholinolytic was the control, and the other samples collected after injection were the test samples. A single injection of the cholinolytic was given.

To determine the concentration of the cholinolytic in the perfusion fluid a calibration curve of the concentration of the drug in the rat's ilium was first plotted. Both the cholinolytic activity of the perfusion fluid and the calibration curve of the cholinolytics were determined by their power to diminish the acetylcholine contracture of the rat's intestine by a given amount.

Control experiments showed that the perfusion fluid alone had no effect on the acetylcholine contracture of the rat's intestine. It should be noted, however, that the sensitivity of the rat's intestine to cholinolytics varies in different animals; in each experiment simultaneous determinations were therefore made of the calibration curve of the cholinolytics and of the cholinolytic activity of the perfusion fluid on the same segments of ilium of the same rat.

EXPERIMENTAL RESULTS

The calibration curve of the diminution of the acetylcholine contracture of the ilium of a rat under the influence of atropine is given in Fig. 1. The broken line shows that the first sample of perfusion fluid obtained after injection of atropine in a dose of 4 mg/kg caused a reduction of 52% of its initial value in the acetylcholine contracture, corresponding to an atropine concentration of 0.39 μg/ml. Since the sample contained 10.2 ml of perfusion fluid, calculation showed that the cholinolytic activity of this sample of perfusion fluid was equivalent to 4.1 μg of atropine. Hence, knowing the concentration of cholinolytic in the sample of perfusion fluid, and the volume of fluid in the sample, the amount of cholinolytic in the sample of perfusion fluid could be determined.

The results of an investigation of the passage of amizil, atropine, and lachesine through the BBB are given in Fig. 2. The height of the columns corresponds to the concentration of cholinolytics in the first samples of perfusion

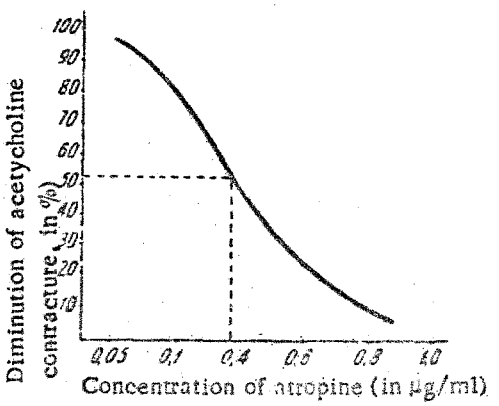


Fig. 1. Calibration curve of diminution of acetylcholine contracture of the rat's ilium after administration of atropine.

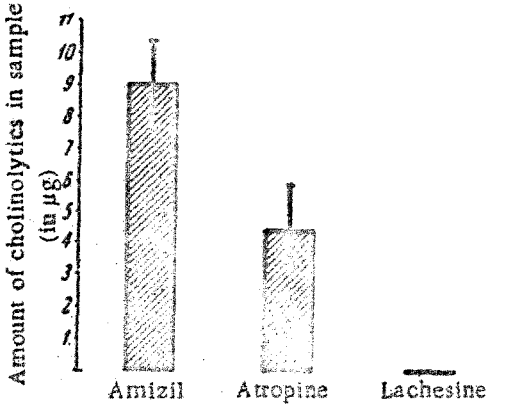


Fig. 2. Passage of cholinolytics through the BBB of the cat after their injection into the carotid artery in a dose of 4 mg/kg.

fluid obtained 30 min after their injection in a dose of 4 mg/kg, determined from the calibration curve. When only one injection of this dose of the cholinolytic was given, the drugs were found only in the first sample of perfusion fluid, whereas in subsequent samples they could not be detected. It is clear from Fig. 2 that, after injection of the cholinolytics into the animals in a dose of 4 mg/kg, the mean concentration of amizil in the first sample of perfusion fluid was 9.0 μg (± 0.35), and of atropine 4.6 μg (± 0.26). No lachesine could be detected in any of the samples of perfusion fluid. Only after injection of lachesine in large doses, such as 40 mg/kg, could it be found in the first sample of perfusion fluid, in a mean amount of 8.3 μg (± 0.32).

It is clear from these findings that lachesine, which contains a quaternary nitrogen atom in its molecule, passes much less readily through the BBB than amizil and atropine, whose molecules contain a tertiary nitrogen atom. These results are in full agreement with those obtained by other workers [1, 2, 5, 10].

It was found, however, that the tertiary compounds also differ in their ability to pass through the BBB. For instance, amizil passes through the BBB approximately twice as easily as atropine. These results agree with those obtained by A. T. Selivanova [8], who showed by a conditioned-reflex method that amizil has a more marked central cholinolytic action, atropine far exceeds amizil. Consequently, the more marked central cholinolytic action of amizil by comparison with atropine may be explained by the fact that it passes more easily through the BBB than atropine.

The results show that differences in passage through the BBB exist not only between the tertiary and quaternary cholinolytics, but also between individual tertiary cholinolytics, and these must be borne in mind when their effect on the central nervous system is studied.

SUMMARY

A method of perfusion of cerebral ventricles was used to study the penetration of cholinolytics through the blood-brain barrier (BBB) of cats. 30 min after injecting cholinolytics in a dose of 4 mg/kg into the carotid artery the average penetration through the BBB of amizil was 9 μg (± 0.35) and of atropine – 4.6 μg (± 0.26), whereas lachesine did not penetrate through the BBB at all. These data indicate that there is a difference in penetration through the BBB not only between the tertiary and quaternary cholinolytics, but also between various tertiary cholinolytics.

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