THE DIFFERENCE IN THE EFFECTS OF TERTIARY AND QUATERNARY AMMONIUM BASES (PROSERINE, SERINE, METHYLATROPINE AND ATROPINE) DEPENDING ON THE METHOD OF ADMINISTRATION

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Schweitzer and Wright [17] discovered in 1937 that certain of the central effects produced by proserine (neostigmine) and eserine (physostigmine), typical "anticholinesterase" agents, in animals differ essentially: proserine inhibits spinal reflexes and prevents strychnine convulsions while eserine enhances these reflexes and has no effect on the course of strychnine poisoning. A number of authors [2, 9, 10] have recently proposed that the central effects of these agents are different because the blood-brain barrier of animals is less permeable by proserine than by aserine; proserine, a quaternary nitrogen derivative, being less soluble in lipids than eserine, which is a tertiary nitrogen derivative. They also believe this to be the reason for the difference in the effects of tertiary and quaternary cholinolytics.

In this work, we studied the effects produced by proserine and eserine and how these compounds are tolerated when intraventricularly administered (which excludes the influence of the blood-brain barrier), as well as when subcutaneously and intravenously administered. We also determined the differences in the effects produced by atropine and its quaternary analog, methylatropine (Eumydrin).

EXPERIMENTAL

The work was performed on male mice weighing 18-22 g each. Aqueous solutions of the experimental substances were introduced under the skin of the back (in a dose of 0.1-0.3 ml), into the caudal vein (in a dose of 0.1-0.2 ml given at a rate of 0.01 ml per sec) or intracranially (in a dose of 0.01-0.03 ml). The intracranial administration was done according to Blume's method [7]. This method specifies that the needle be inserted 2 mm deep at a point 3 mm anterior and 2 mm lateral to the chiasm of the sagittal and lambdoid sutures of the mouse's cranium. The solution is injected into the lateral ventricle of the brain. To facilitate manipulation, the mice were put under ether anesthesia. The control experiments showed that anesthetization of the mice did not affect the subsequent course of poisoning or the lethality level. The median lethal doses computes with G. N. Pershin's formula [3], Miller and Tainter's method was used to determine the average error [17], and Fisher's table [14] was used to determine the significance of the differences we detected in the average levels. We used proserine methylsulfate, eserine salicylate, atropine sulfate and methylatropine nitrate [atropine methylnitrate].

RESULTS

Dyspnea and fascicular muscular twitching were observed in mice soon after the subcutaneous injection of proserine, followed by tremor (kyperkinesia of central origin) and profuse lacrimation and salivation. Attacks of strong clonic convulsions developed after a few minutes. Paresis of the posterior extremities developed in the mice after the first 2-3 attacks; the respiration became retarded, changed into single sighs, then ceased.

The first sign of poisoning by subcutaneously administered eserine was a typical tonic convulsion of the tail. Tremor and salivation were more pronounced than with proserine intoxication. The convulsions were clonicotonic from the very beginning. The mice remained able to move about in the periods between convulsive attacks. Paresis of the extremities was never observed. Most of the animals died at the height of a convulsive attack.

The intravenous administration of proserine and eserine considerably accelerated the course of the poisoning. Convulsions developed immediately after the proserine injection and after a very short latent period in eserine poisoning. In both cases, the convulsions were stronger than those induced by the subcutaneous injections; the convulsions which developed in proserine poisoning were purely clonic, while those observed with eserine poisoning had a tonic component. Death usually occurred at the height of the convulsions.

After the intracranial administration of proserine, most of the animals did not budge for several minutes from the lateral position induced by the ether administered before the injection; non-anesthetized mice administered proserine in this manner took a lateral position shortly after the injection. Peripheral symptoms of poisoning (salivation and fasciculation) developed in the mice one minute after the injection, but these were considerably less pronounced than those observed after subcutaneous or intravenous administration. The clonic convulsions which then developed in 90% of the mice were milder than those induced by the subcutaneous injection and much milder than those induced by the intravenous injection. The tremor was so mild that it was impossible to fix the time of its onset. Stupor similar to that described by Feldberg [13] in cats intraventricularly injected with acetylcholine and diisopropyl fluorophosphate developed in some of the mice, particularly those administered the absolute lethal dose; death resulted from respiratory arrest.

TABLE 1. LD_{50} (in mg/kg weight) and Average Time after Which Convulsions and Death Occurred (in min) in Mice Administered the Experimental Substances in Different Ways

Substance	Index	Administration method		
		subcutaneous	intravenous	intracranial
Proserine	LD_{50}	0.40 ± 0.04	0.22 ± 0.01	0.14 ± 0.06
	Time convulsions developed	6.2 ± 0.1	Instantly	4.4 ± 0.3
	Time death occurred	13.0 ± 0.2	2.5 ± 0.1	7.2 ± 0.3
Eserine	LD_{50}	0.84 ± 0.03	0.54 ± 0.02	0.95 ± 0.04
	Time convulsions developed	6.8 ± 0.3	0.7 ± 0.02	1.9 ± 0.1
	Time death occurred	11.5 ± 0.3	3.8 ± 0.4	4.8 ± 0.2
Methyl-atropine	LD_{60}	242.0 ± 12.0	21.0 ± 4.0	6.8 ± 0.18
	Time convulsions developed	5.8 ± 0.5	Instantly	9.9 ± 0.4
	Time death occurred	60 – 240	4.1 ± 0.2	60 – 180
Atropine	LD_{50}	578.0 ± 30.0	70.0 ± 4.0	5.9 ± 0.2
	Time convulsions developed	None	Instantly	8.0 ± 0.3
	Time death occurred	300 – 500	4.6 ± 0.2	51.0 ± 3.

When eserine was intracranially administered, all the mice moved out of the lateral position soon after the injection and ran around the jar. The same peripheral symptoms of poisoning were observed as with the intracranial administration of proserine. Convulsions developed in only 20% of the animals and were purely tonic in nature. Symptoms of acute respiratory disorder were followed by death.

In the experiments with atropine and methylatropine, we also observed differences in the symptoms of intoxication, whether these substances were administered by the same method or by different methods.

The symptomatology of the poisoning picture resulting from the subcutaneous injection of atropine was meager. In most cases, convulsions either did not develop at all or were very mild. A long period of depression of respiration and motor activity began 20-30 min after the injection and ended in death. Strong clonic convulsions distinguished methylatropine poisoning from atropine poisoning. The convulsive period was succeeded by inhibition of the central nervous system.

Clonic convulsions developed immediately after the intravenous administration of atropine and methylatropine. The poisoning induced by these two substances evidently followed the same course.

TABLE 2. Symptoms of Proserine, Eserine, Methylatropine and Atropine Poisoning in Mice with Different Methods of Administration

Substance	Administration method														
	subcutaneous					intravenous				intracranial					
	salivation	tremor	force of convulsions	type of convulsions	paresis of extremities	salivation	tremor	force of convulsions	type of convulsions	termination of anesthesia	salivation	tremor	force of convulsions	type of convulsions	stupor
Proserine	+++	++	++	Clonic	Yes	++	++	+++	Clonic	Retarded	+	±	+	Clonic	Yes
Eserine	++++	++++	++	Cloni-cotonic	No	+++	+++	+++	Cloni-cotonic	At once	+	+	±	Tonic	No
Methylatropine	No	No	++	Clonic	No	No	No	+++	Clonic	At once	No	No	+++	Clonic	No
Atropine	No	No	No	No	No	No	No	+++	Clonic	Retarded	No	No	++++	Clonic	No

Note: Crosses show relative degree to which symptoms were expressed: (±) – symptom barely apparent; (+) – symptom mild; (++) – average; (+++) – strong; (++++) – very strong.

With intracranial administration of atropine, the mice did not budge from the lateral position until several minutes after the injection, after which they spun around on the longitudinal axis of their bodies and tried to run. Right after this, they developed extremely strong clonic convulsions, lasting 30 min or more. Most of the animals died at the height of these convulsions. The mice abandoned the lateral position very quickly after the administration of methylatropine; convulsions were quicker to develop, but milder and less lasting than those observed with atropine poisoning. Only a few of the mice died at the height of the convulsions. In most of the animals, the convulsive period was succeeded by a period of depression.

Table 1 gives the average lethal doses, average time required for the development of convulsions and average time of death for the various administration methods.

As the investigations conducted showed, the effects produced by intracranial administration of proserine, eserine, methylatropine and atropine differ substantially from those produced by subcutaneous and intravenous administration of these substances. Moreover, intracranially administered proserine produces effects which differ qualitatively from those induced by intracranially administered eserine, just as the effects induced by methylatropine differ from those induced by atropine. Table 2 contains data characterizing the symptoms of poisoning induced by these four preparations. Since the qualitative differences in the pictures of poisoning caused by tertiary and quaternary compounds persist when the substances are introduced into the ventricles of the brain, one can assume that these differences are not due solely to the different permeability of the blood-brain barrier to these compounds, as a number of authors believe. The validity of the reason given for the difference in the central effects of tertiary and quaternary compounds, i.e. that the blood-brain barrier is less permeable by compounds poorly soluble in lipids, is especially doubtful in the case of mice. The fact that the tolerance of the mice for these substances differed depending on the method of administration does not permit one to concede that the blood-brain barrier of these animals is less permeable by quaternary bases such as proserine and methylatropine than by compounds such as eserine and atropine, which contain tertiary nitrogen. This viewpoint cannot account for the following facts: 1) subcutaneously administered, methylatropine is more than twice as toxic as atropine, although death caused by poisoning with either preparation results from injury of the central nervous system; 2) with intracranial administration, atropine tolerance decreases (as compared with subcutaneous administration) three times as much as methylatropine tolerance and 35 times as much as proserine tolerance; 3) the quaternary ammonium base tubocurarine, which induces peripheral paralysis in most animals, in mice, causes clonic convulsions and death resulting from paralysis of the respiratory center [1].

The difference in the central symptoms of poisoning caused in mice by proserine and eserine we propose to be due to the fact that proserine causes greater stimulation of the n-cholinergic systems than eserine does [4]. According to this hypothesis, the clonic nature of the convulsions which occur in proserine poisoning is due to the fact that proserine stimulates primarily the regions containing mainly n-cholinergic neurons, i.e. the cortex and adjacent subcortical formations [11]. The tonic component of the convulsions and the strong tremor observed in eserine poisoning are probably associated with excitation of the thalamus and the reticular formation of the midbrain – regions containing chiefly m-cholinergic neurons [8, 12], stimulation of which can reproduce both these symptoms [6, 15].

There is no doubt that in the case of other animals as well as mice (in which the blood-brain barrier is apparently permeable by organic cations), the differences in the central effects of tertiary and quaternary compounds cannot be ascribed solely to the different permeability of the blood-brain barrier to these compounds. On the basis of data indicating the high sensitivity of the caudal sections of the reticular formation of cats to quaternary cholinolytics [5, 14] and the results we obtained, one can assume that the difference in the effects is due mainly to the elective reaction of the various sections and neurons of the brain to these two types of compounds.

SUMMARY

Differences in the effects produced in mice by tertiary and quarternary ammonium compounds are observed not only after their subcutaneous injection, but also when administered into the cerebral ventricles.

The tolerance of mice to methylatropine is lower than to atropine, and in passing over from the subcutaneous to intracerebral route of administration it diminishes to a lesser extent. It is suggested that hematoencephalic barrier of mice is permeable to quarternary ammonium compounds. The differences in the central effects of the tertiary and quaternary ammonium bases in animals should not be attributed to the varying hematoencephalic barrier.

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