DIFFERENTIATION OF THE PHARMACOTHERAPEUTIC EFFECTS OF RESERPINE AND SEROTONIN IN ADULT AND NEWBORN MICE POISONED WITH AMETHOCAINE

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UDC 615.781.63+615.781.63-053.31]-099.085.7

Many reports of the influence of local anesthetics on the activity of cholinergic structures have been published [2-5, 7-9, 11, 13, 19, etc.]. However, much less is known of their action on the activity of adrenergic or serotoninergic structures [1, 6, 19, etc.].

In the present investigation the effect of reserpine, serotonin, and adrenalin was studied on the toxic manifestations of amethocaine in adult and newborn animals.

EXPERIMENTAL METHOD

Experiments were conducted on 182 adult and 90 newborn mice.

Experiments on adult animals. Series I: reserpine (2 μg/g body weight) was injected intraperitoneally twice daily for 2 days; on the 3rd day the last injection of reserpine was given and was followed 15 min later by the subcutaneous injection of amethocaine. Series II: reserpine (0.1-1.0 μg/g body weight) was injected once, 1 h before the animal received amethocaine. Series III: serotonin (10-20 μg/g body weight) was injected intraperitoneally 15 min before injection of amethocaine. Series IV: serotonin (0.5-5.0 μg per mouse in 0.02 ml of solution) was injected into the lateral ventricle of the brain 10 min before injection of amethocaine.

The intraventricular injection was given as follows. The mouse was anesthetized with ether, the skull was exposed, and at the moment when the animal came round from the anesthetic the injection was given by a needle with a guard to a depth of 2 mm at a point 3 mm anteriorly to the lambdoid suture and 2 mm laterally to the sagittal suture. For verification purposes the serotonin solution was colored with methylene blue. After death of the mouse the ventricles of the brain were exposed and the accuracy of injection of the preparation judged from their staining (surviving animals were sacrificed and the correctness of injection of the preparation was also verified). Amethocaine was injected subcutaneously into all the animals in the form of a freshly prepared 0.5% aqueous solution in a dose of 45-50 μg/g body weight, causing death of 60-85% of the mice.

In series V, adrenalin (0.2 μg/g body weight) was injected intraperitoneally 15 min before the animals received amethocaine.

Experiments on newborn mice. Amethocaine was injected subcutaneously into animals 2 or 3 days old in the form of a 0.8% solution in a dose of 80-88 μg/g body weight. Reserpine, in a dose of 0.1 or 1.0 μg/g body weight, was injected into the animals intraperitoneally, 1 h before injection of the amethocaine. Serotonin (10-20 μg/g body weight) or adrenalin (0.2 and 1.0 μg/g body weight) was injected intraperitoneally 15 min before the injection of amethocaine. During the experiment the mice were kept at 26-27°. Observations were kept on the animals for 3-3½ h.

EXPERIMENTAL RESULTS

In the experiments in which a preliminary course of reserpine was given (series I), no difference was observed from the controls (5 of 6 experimental mice and 3 of 6 control mice died).

When a single injection of reserpine was given 1 h before the injection of amethocaine (Table 1), the mortality of the mice from amethocaine poisoning decreased. This effect was observed only if small doses (0.1 μg/g body weight) of reserpine were given ($P < 0.01$).

Department of Pharmacology, Leningrad Pediatric Medical Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR S. V. Anichkov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 7, pp. 64-67, July, 1966. Original article submitted July 27, 1965.

TABLE 1. Effect of Reserpine, Injected 1 h before the Experiment, on the Outcome of Amethocaine Poisoning in Adult Mice

Reserpine (in μg/g body weight)	Amethocaine (in μg/g body weight)	Control	Experiment
0.1	50	17/20	8/20
0.25	50	8/10	4/10
0.1	50	8/10	5/10
1	50	8/10	9/10

Note: Here and in Tables 2-4, denominator—number of animals in experiment, numerator—number of dying animals.

TABLE 3. Effect of Intraventricular Injection of Serotonin on Outcome of Amethocaine Poisoning in Adult Mice

Serotonin	Amethocaine	Control	Experiment
in μg/g body weight			
5	50	3/5	5/5
1	45-50	9/15	9/15
0.1	50	3/5	4/5

TABLE 2. Effect of Intraperitoneal Injection of Serotonin and Adrenalin on Outcome of Amethocaine Poisoning in Adult Mice

Preparation tested (in μg/g body weight)	Amethocaine (in μg/g body weight)	Control	Experiment
Serotonin:			
20	50	7/10	5/10
10	50	7/10	6/10
Adrenalin:			
0.2	50	8/10	8/10

In the experiments in which the animals first received serotonin or adrenalin, and in which serotonin was injected intraventricularly into adult mice, these substances were found to have no effect on the outcome of amethocaine poisoning (Tables 2 and 3).

In the newborn mice the intraperitoneal injection of reserpine had no effect on the mortality from amethocaine poisoning, in contrast to its action on adult animals (Table 4). On the other hand, the intraperitoneal injection of serotonin had an obvious prophylactic effect in the newborn mice, preserving the life of nearly all the animals after receiving doses of amethocaine causing death of 70% of the control mice (see Table 4). Injection of adrenalin did not prevent death of the young mice (see Table 4).

It follows from these results that the injection of a small dose of reserpine 1 h before injection of amethocaine helped to secure survival of adult animals poisoned with amethocaine. On the other hand, the injection of large doses of reserpine (a single injection or course of injections) gave no such effect. An important link in the mechanism of action of reserpine is the weakening of the bond between the catecholamines and serotonin, on the one hand, and the reserve proteins [13] on the other. In these circumstances they are at first liberated profusely, leading to a temporary increase in the content of these substances in various tissues, including the brain. It may be postulated that the prevention of death of the mice poisoned with amethocaine by injection of reserpine into the animals 1 h before the experiment was associated with the increase in the content of catecholamines or serotonin in the central nervous system.

The absence of a protective effect of reserpine in the newborn animals is evidently the result of a low content of biogenic amines in the body of these animals [15, 16, 17].

As the results described above show, adrenalin did not lower the mortality among the animals from amethocaine poisoning. Nor did serotonin prevent death of the adult mice when administered by different routes. In the newborn mice, on the other hand, serotonin, injected intraperitoneally, gave a well marked prophylactic effect. This observation demonstrates the antagonism between serotonin and amethocaine. Antagonism between local anesthetics and serotonin has been reported earlier [6]; it has been observed that this antagonism is proportional to the strength of the anesthetic action of the preparations. The author has found that serotonin prevents death from amethocaine poisoning only in newborn animals. The extensive experimental literature shows that in newborn animals the blood-brain barrier does not function adequately and permits many endogenous and exogenous substances to reach the brain which, in the adult animal, either do not reach the brain tissue at all or reach it in small quantities [10, etc.]. Serotonin likewise does not penetrate into the central nervous system of adult animals if it is inactivated by the monoamine oxidase present in the walls of the brain capillaries [19]. Even when serotonin is injected into the cerebral ventricles and enters the cerebrospinal fluid, it evidently does not penetrate deep into the

TABLE 4. Effect of Intraperitoneal Injection of Reserpine, Serotonin, and Adrenalin on the Outcome of Amethocaine Poisoning in Newborn Mice

Preparation tested (in μg/g body weight)	Amethocaine (in μg/g body wt.)	Control	Experiment
Resperine			
0,1	80—88	8/10	8/10
1	80—88	8/10	8/10
Serotonin			
10	80—88	7/10	1/10
20	80—88	7/10	0/10
Adrenalin			
0,2	80—88	8/10	8/10
1	80—88	8/10	5/10

brain tissue (this assumption is quite acceptable, for histamine, when injected into the cerebral ventricles, penetrates into the brain to a depth of only 3 mm [14]).

It may be concluded from these results that the prevention of the toxic effect of amethocaine in newborn mice by serotonin is the result of their central antagonism. The results demonstrate the possibility that pharmacotherapeutic effects may be obtained in newborn animals which are not found in the adult.

SUMMARY

The object of study was the influence of preliminary injections of reserpine, serotonin and adrenalin on the course of amethocaine intoxication in adult and newborn mice. It was found that in adult animals reserpine, rather than serotonin or adrenalin, averted death due to amethocaine intoxication. In newborn animals, however, reserpine proved ineffectual, while serotonin was 100% effective.

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