EDEMA OF THE LUNGS IN ALBINO RATS AND RABBITS AFTER INJECTION OF ACONITINE INTO THE HYPOTHALAMUS

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The commonest causes of pulmonary edema in man are certain diseases and operative and traumatic injuries of the central nervous system [2, 3, 6, 15]. This type of edema has been produced experimentally by injury to the skull by compression, by blast, by injection of various irritant substances into the cisterna magna of the brain, and by injury to the brain with a probe [4, 5, 7, 8, 10]. The production of pulmonary edema by accurately localized electrolytic injury to the preoptic zone of the hypothalamus in rats [11, 12] and dogs [15], and of pulmonary edema in rats by injection of a very dilute solution of aconitine [14], is particularly interesting. The principal and magnocellular nuclei of the preoptic zone in the dog, according to Urabe [15], act as the highest sympathetic vasomotor center for the pulmonary circulation. The regulation of autonomic functions, including vaso-tissue permeability [1], is concentrated in the region of the hypothalamus. To study the pathogenesis of the various types of centrogenic edema and to understand the central nervous mechanisms of pulmonary edema of different origin, it is extremely important to be able to reproduce edema in animals of different species, arising as the result of accurately localized action on the hypothalamus, and to study the principles governing its development.

In this paper the authors describe the results of experiments on rats and rabbits in order to produce pulmonary edema by injecting aconitine into the preoptic region.

EXPERIMENTAL METHOD

Experiments were carried out on 105 noninbred rats weighing 170-330 g and on 16 rabbits weighing 1400-2450 g. For comparison, the lungs of 57 healthy rats and 25 rabbits were used. Aconitine hydrochloride, in a dilution of $1:10^5$ (for rats) and $1:10^3$ (for rabbits) was injected through a fine injection needle of a tuberculin syringe fixed in the electrode holder of a stereotaxic apparatus. The rat's head was held in a type STM apparatus of the Institute of Higher Nervous Activity, Academy of Sciences of the USSR, using the Groot's atlas [9]. Injections were given alternately at 2 or 3 points: 0.5-1.0 mm on either side of the midline of the brain and in the midline in the planes A-7.6-8 at a depth of 1.5 mm from the base of the brain. The experiments on rabbits were carried out by means of the apparatus of the A. A. Bogomolets' Institute of Physiology, Kiev, using the coordinates of Sawyer's atlas [13]. Aconitine was injected into three points: along the midline and 1 mm from it on either side in the planes A-3.5-4.5, to a depth of 1.5-2 mm from the base of the brain.

The dying rabbits were autopsied, the ratio between the weight of the lungs and the body weight was determined in percent (the pulmonary coefficient—p.c.), and after drying to constant weight at 80-90°, the dry residue (d.r.) of the lung tissue was calculated as a percentage of the weight of the fresh tissue. In many animals the lung tissue was examined histologically. The brain of the dying rats and rabbits was again fixed in the stereotaxic apparatus and the brain was marked by electrocoagulation in planes parallel to the planes of injection of aconitine. After removal of part of the skull, the brain was fixed in 10% formalin, and then carefully removed, sliced along the planes of the mark, and sections were cut to a thickness of 90-120 μ on a freezing microtome. Sections in which injury by the needle could be seen were placed on a glass slide, and negative photographs obtained on photographic paper by means of an enlarger. By comparing these with the plates of the atlases, the site of injection of the aconitine could be determined.

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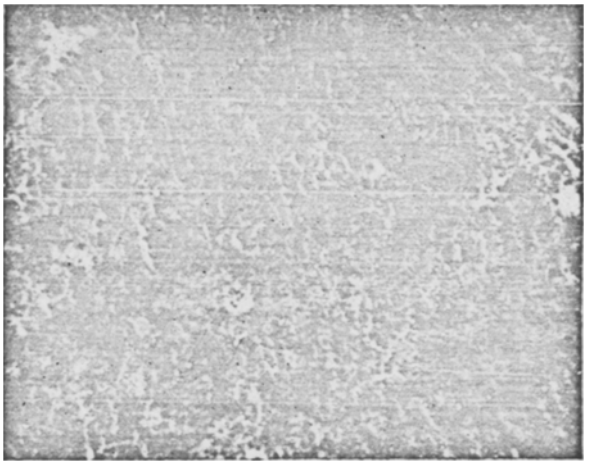

Fig. 1. Lungs of a rat after injection of aconitine into the hypothalamus. Severe edema with massive hemorrhages (p.c. 1.71; d.r. 13-55%). Hematoxylin-eosin. Objective 20×, ocular 10×.

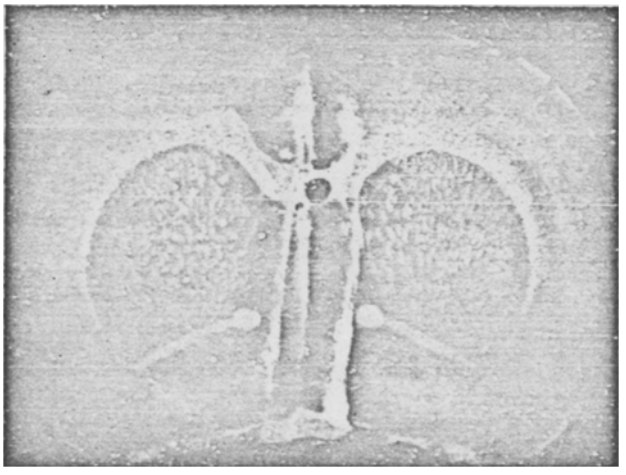

Fig. 2. Section through a rat's brain in the plane A-7.8 after injection of aconitine into the hypothalamus. Hemorrhages can be seen along the course of the three channels of the injection needle and in the lateral ventricles. Magnification 7×.

EXPERIMENTAL RESULTS

Rats. In most experiments after the first injection of aconitine, a convulsive reaction appeared, and respiration quickened considerably and became paroxysmal. After the end of the injections the respiration became slower and deeper and the breath was held at the height of inspiration. Cyanosis of the snout, ears, and paws appeared. After injection of hexobarbital, the body temperature of all the rats fell (in some experiments to 24-26°). In three rats, surviving a few hours after injection of aconitine, the fall of temperature was replaced by a rise to 0.5-2° above its initial level. In five animals, also surviving for a fairly long time, signs of aggressiveness developed, which, like hyperthermia, indicate excitation of the anterior portions of the hypothalamus [11]. Before death, which took place from respiratory arrest, clonic and tonic convulsions frequently developed and a blood-stained froth escaped from the nose.

For the analysis of the experimental results, most of the rats (83) were included in the group with pulmonary edema; 74 of these rats died 5-89 min after the beginning of the injection, and in most cases (54 rats) death followed after not more than 15 min. Two rats were sacrificed after 4 h and 11 h 15 min.

Pulmonary Coefficient and Dry Residue of Lung Tissue of Healthy Rats and Rabbits after Injection of Aconitine into the Hypothalamus

Series of Animals	No. of Animals	p.c.	d.r.
		M ± m	
Rats			
I (healthy)	57	0,58±0,016	21,41±0,17
II (with pulmonary edema)	83	1,60±0,015 $P<0,001$	13,53±0,19 $P<0,001$
III (without pulmonary edema)	22	0,64±0,087	20,81±0,09 $P<0,01$
Rabbits			
I (healthy)	25	0,36±0,033	21,30±0,24
II (with pulmonary edema)	14	0,87±0,085 $P<0,001$	15,63±0,15 $P<0,001$
III (without pulmonary edema)	2	(0,39 и 0,36)	(19,77 и 20,63)

Note. The values of P are given when the difference from series I is significant.

At autopsy the lungs were covered with large, livid stains and occupied the whole of the thorax. In some cases the lungs were enlarged but pale in color, with petechial hemorrhages. The trachea was filled with blood-stained frothy liquid, which also effused from the surface of the incision. In some rats fluid was found in the pleural cavity. Under the microscope the alveoli and interalveolar septa were filled with edema fluid and erythrocytes and the blood vessels were congested (Fig. 1). The gravimetric p.c. in the rats of this group was almost doubled because of the congestion and edema, while the value of d.r. was considerably reduced, confirming the accumulation of the large excess of fluid in the lungs. Both indices showed a statistically significant difference from the corresponding values in the healthy rats (see table, series II).

Edema did not develop in 22 rats of series III. They died as a rule later than the animals with pulmonary edema: between 10 min and 15 h 30 min after; of the 11 rats which died, only 5 died after 10-24 min. The other 11 animals were sacrificed 4-24 h after the injection. In many of the rats of this series the Nembutal or hexobarbital anesthesia was deeper than the anesthesia in the 83 animals with pulmonary edema, most of which were only superficially anesthetized with hexobarbital. Autopsy of the rats without edema showed that their lungs usually differed only slightly from the lungs of healthy animals. In some experiments they were slightly congested, with petechial hemorrhages on the surface. Histological investigation sometimes revealed congestion and edema in solitary alveoli. The value of p.c. was higher on the average than for healthy animals, but the difference was not significant, (see table, series III). The accumulation of an excess of water in the lungs, despite the absence of frank edema, was proved by the significant decrease in the weight of the dry residue.

The study of brain sections of rats with or without pulmonary edema showed that the localization of the aconitine injections was the same in both. In the great majority of experiments the injection needle passed through the planes A-7.8-8 along the midline and on both sides of it, reaching as far as the optic chiasma. Deviations in all three planes were observed in a few rats of both series. Nearly always hemorrhages were seen in the third and lateral ventricles (Fig. 2).

Rabbits. All 16 animals were lightly anesthetized with hexobarbital. In connection with this their body temperature fell only to 34.5-35°. Injection of aconitine was accompanied by a convulsive reaction, an increase followed by a decrease in the respiration rate, deepening respiration, and breath-holding in inspiration. Thirteen rabbits had convulsions before death and a blood-stained froth escaped from the nose; ten animals died 6-22 min after the injection, 6 survived for between 41 min and 1 h 42 min. Because of the early death of the rabbits, they did not develop hyperthermia or aggressiveness.

At autopsy of two rabbits small, pale pink lungs were found with normal values of p.c. and d.r. (see table, series III). In the remaining 14 animals obvious external signs of edema were seen, with values of

p.c. and d.r. differing from the corresponding values in healthy animals (series II) by a highly significant amount. It was noted that in half the rabbits with pulmonary edema, it developed against the background of severe congestion with a large increase of weight (mean p.c. 1.3) and with a considerable decrease in dry residue (mean d.r. 14.07%). The remaining 7 animals also showed undoubted signs of edema (mean d.r. 16.66%). However, their lungs were pink with petechial hemorrhages. The absence of marked congestion of the lungs was confirmed by the moderate increase in their weight (mean p.c. 0.55). The development of edema after injection of aconitine into the hypothalamus, although taking place (especially in rats) against the background of marked congestion of the lungs, may also occur without significant disturbances of the pulmonary circulation. Examination of the brain sections showed that 9 of the 14 rabbits with pulmonary edema and one rabbit with pulmonary edema and one rabbit without edema received the aconitine injection in planes A-3.5-4, passing in nearly all cases through the midline as far as the optic chiasma. In 5 rabbits with edema and 1 without the needle deviated in an antero-posterior direction. In nearly all the rabbits hemorrhages were found in the third and lateral ventricles.

Hence, after injection of aconitine solution into the hypothalamus of rats and rabbits, no strict relationship was found between the localization of the point of the injection needle and the presence or absence of pulmonary edema. In earlier experiments with electrolytic injury of the same parts of the brain in rats and rabbits a regular relationship was found between the topography of injury and the development of edema, the frequency of which was much less than in the experiments with aconitine. Introduction of the needle into the rat's brain in the planes A-7.8-8 or in the analogous planes A-3.5-4 of the rabbit's brain, in relation to the coordinates chosen, was always accompanied by puncture of the ventricles with escape of blood into them. It may be assumed that, besides blood, part of the injected aconitine penetrated into the cerebrospinal fluid system of the brain along the channel of injection, causing the development of pulmonary edema, by stimulating certain areas of the brain bounding the cavity of the ventricles. This problem, and also the specificity of the edemogenic action of aconitine, will be examined in a continuation of this investigation.

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