CATECHOLAMINE LEVELS IN THE BLOOD, HEART, AND ADRENALS OF RATS AFTER INTRAPERITONEAL INJECTION OF A CARDIOTOXIC DOSE OF NORADRENALIN PRECEDED BY ARFONAD AND RAUSEDIL

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In cardiovascular pathology and, in particular, under conditions of stress a change in the state of the sympathico-adrenal system leads to the development of disseminated focal injuries to the heart.

However, the role of a change in the concentrations of catecholamines (CA) in the blood and myocardium in this process remains unexplained. Considering that CA for several minutes after administration can have a toxic action and undergo metabolic conversions [3], it was decided to study the dynamics of these substances in the blood, myocardium, and adrenals during the first minutes after intraperitoneal injection of noradrenalin (NA), after preliminary injection of Arfonad (a ganglion-blocker) and Rausedil (a reserpine preparation), and also to compare the CA level in the above tissues with the degree of myocardial damage.

EXPERIMENTAL METHODS

Four series of experiments were carried out on 74 male Wistar rats weighing 200-250 g. In series I the rats were killed by decapitation 45 sec and 2 and 7 min after intraperitoneal injection of NA (2.5 mg/kg). Since under these circumstances the greatest fluctuations in the CA level in the blood and heart were observed 2 min after injection of NA, in all the other experiments the animals were killed at that time. In series II, 7 min before injection of NA, the rats were given an intraperitoneal injection of Arfonad (5 mg/kg), and in series III they were given an injection of Rausedil (4 mg/kg) 24 h before injection of NA. These times of injection of the drugs were chosen so that the toxic action of NA developed at the time of maximal influence of the ganglion-blocker and sympatholytic. In the controls to each series, distilled water was injected instead of NA. Considering that in all the experiments the test drugs were injected twice, with a short interval between them, a control series of experiments was set up in which two injections of distilled water were given and the animals were killed 2 min after the second injection. All the experimental animals were thus distributed among the following groups: 1) intact; 2) two injections of distilled water with an interval of 7 min between them and sacrificed 2 min after the second injection; 3) injection of distilled water, injection of NA 7 min later, and sacrificed after 45 sec; 4) injection of distilled water, injection of NA 7 min later, sacrificed after 2 min; 5) injection of distilled water, injection of NA 7 min later, sacrificed after 7 min; 6) injection of Arfonad, injection of water 7 min later, sacrificed after 2 min; 7) injection of Arfonad, injection of NA 7 min later, sacrificed after 2 min; 8) injection of Rausedil, two injections of distilled water 24 h later, sacrificed 2 min after the second injection; 9) injection of Rausedil, injection of distilled water 24 h later, injection of NA 7 min later, sacrificed after 2 min.

The CA concentration in the heart, adrenals, and blood was determined by a fluorometric method in the modification in [4, 5].

Besides the biochemical investigation, the vulnerability of the myocardium was studied morphologically. For this purpose NA was injected into some of the animals in a dose of 2.5 mg/kg; Rausedil (4 mg/kg) and

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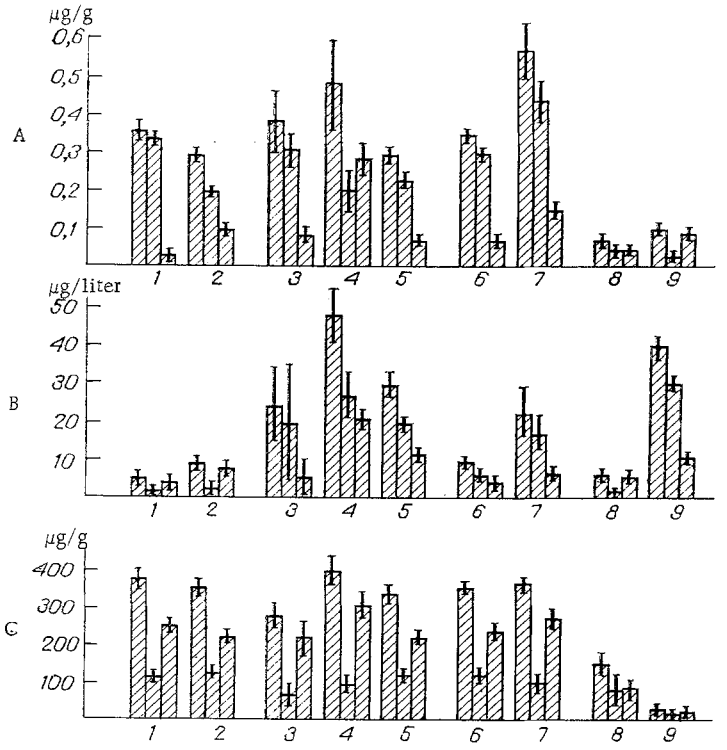

Fig. 1. Concentrations of CA in blood, heart, and adrenals of rats after intraperitoneal injection of NA preceded by Arfonad and Rausedil. A) CA concentration in heart (in μg/g); B) CA concentration in blood (in μg/liter); C) CA concentration in adrenals (in μg/g). Numbers between groups of columns correspond to numbers of groups of experimental animals given in "Experimental Method." First column in group shows total CA (A +NA) concentration, second column NA, third column A concentration. Ordinate, concentration of CA.

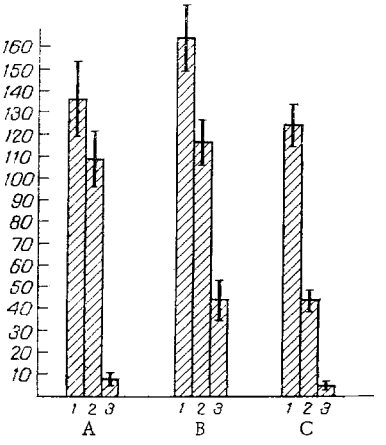

Fig. 2. Number of injured myocytes in heart of rats receiving intraperitoneal injection of NA preceded by Arfonad and Rausedil. A) Number of injured cells in left ventricle (1), ventricular septum (2), and right ventricle (3) after injection of NA. B) The same, after injection of NA preceded by Rausedil; C) the same after injection of NA preceded by Arfonad. Ordinate, mean number of damaged cells per section.

Arfonad (5 mg/kg) were injected, like the NA, intraperitoneally 24 h and 7-10 min respectively before the injection of NA. The rats were decapitated 6 h after injection of NA. The hearts were quickly removed and fixed in 10% buffered formalin, after which sections were cut in the frontal plane and embedded in paraffin wax. Sections 5-7 μ thick were stained by the method of Lie et al. [9]. Injured myocytes, which were stained bright red, were counted in the ventricles and ventricular septum in each fifth serial section, five sections being examined in each observation. In each series of the morphological investigation ten animals were studied.

EXPERIMENTAL RESULTS

The results of the biochemical investigation are given in Fig. 1. Their analysis showed that the NA content in the myocardium of the intact animals was 15 times greater than that of adrenalin (A) ($P < 0.001$), whereas in the blood, on the contrary, the concentration of A was four times higher than that of NA ($P < 0.01$). Two intraperitoneal injections, regardless of the substance injected, significantly changed the CA concentrations in the blood and myocardium of the rats, as shown by an increase in the A concentration in the blood and myocardium by 2 and 5 times respectively ($P < 0.01$). This phenomenon evidently is associated with liberation of CA (predominantly A) from the adrenals followed by uptake of the hormones by corresponding tissues. Preliminary injection of Arfonad considerably reduced the liberation of CA from the adrenals and the degree of increase in the A concentration in the myocardium.

The blood NA concentration was increased tenfold ($P < 0.001$) after only 45 sec, whereas its concentration in the adrenals was reduced by half ($P < 0.01$), but, as already mentioned, the greatest changes in the CA level in the tissues studied were observed after 2 min. At that time the significant increases were observed in the concentrations of A (by 2.6 times; $P < 0.01$) and NA (by 20 times; $P < 0.001$) in the blood and in the A concentration in the myocardium (by 2.8 times; $P < 0.01$). Most indices returned to their initial levels after 7 min, but the NA concentration in the blood, although it was reduced, still remained higher (by 10 times; $P < 0.001$) than in the control. Injection of NA after Arfonad did not result in any increase in the blood A level, but was accompanied by an increase in the NA concentration in both the blood and the myocardium. These results are evidence that intraperitoneal injection of NA increases the CA concentration in the blood and myocardium on account of both endogenous hormones (mainly A) and of exogenous NA. The smaller rise in the A concentration in the blood and myocardium after administration of Arfonad is in agreement with observations of Glants [2], who showed that injection of ganglion-blockers prevents the response of the adrenal medulla to stimulation of interoceptors.

The A concentration in the heart and adrenals 24 h after injection of Rausedil was reduced by two-thirds ($P < 0.01$), and the NA concentrations in the blood and heart muscle were reduced by 75 and 84% respectively ($P < 0.01$). Injection of NA after Rausedil increased its blood level by 22 times compared with that observed in the control in which two injections of water were given and by 90 times compared with its level after injection of Rausedil. The NA concentration in the myocardium showed no significant change under these circumstances. The A concentration in the blood and myocardium was increased twofold ($P < 0.01$). These changes in the CA concentration in the blood and myocardium were accompanied by a sharp decrease in their concentration in the adrenals. Just as after preliminary injection of Rausedil, intraperitoneal injection of NA thus led to mobilization of endogenous CA from the adrenals. Since Rausedil does not inhibit neuronal uptake of CA [7], the absence of any increase in the NA concentration in the heart at a time of 90-fold increase in its concentration in the blood can evidently be attributed to depression of storage of the mediator in adrenergic granules during neuronal uptake. In this case, CA is known to be quickly catabolized [1, 10].

When the results of this investigation are assessed as a whole, it will be noted that changes in the CA concentration in the tissues tested were transitory in nature: Changes in the CA concentration were observed as early as 45 sec after injection of NA, and by the 7th minute they were back to the control level. Comparison with the results of a morphological investigation (Fig. 2) shows that the increase in the CA concentration in the blood observed during the first minute after injection of NA was probably an important factor in causing injury to the myocardium, for the decrease in their concentration following ganglion-blockade was accompanied by a decrease in the degree of damage to the ventricular septum and right ventricle by 2.5 and 1.7 times respectively ($P < 0.01$). After administration of Rausedil, injection of NA led to considerably more severe damage to the myocardium of the left and right ventricles, by 1.2 ($P < 0.01$) and 5 times ($P < 0.001$) respectively, without any increase in the NA concentration in the myocardium, whereas the blood CA level rose sharply. The differences in the degree of increase in the A and NA concentrations in the heart muscle and the absence of direct proportionality between the marked increase in the NA concentration in the blood plasma and the small increase in its concentration in the myocardium emphasize the complex relationships existing in vivo between

the uptake and liberation of CA in the heart [1]. Competition between A and NA for combining sites and transport of CA in nerve endings may perhaps play an important role in this effect [6]. It is also known that the hypercatecholaminemia, causing subsensitivity of presynaptic α-adrenoreceptors, leads to increased liberation of the sympathetic mediator from the nerve endings and, consequently, to a decrease in its content in the sympathetic nerves [8]. It will be clear from the above remarks that to assess the degree of injury of the myocardium and to judge its pathogenesis purely by the CA concentration in the heart is without basis.

The results of this investigation thus show that during the first minutes after intraperitoneal injection of NA the CA concentration in the blood rises considerably. The hypercatecholaminemia which develops is evidently an important factor in the pathogenesis of the focal lesions of the myocardium, but the NA concentration in the myocardium does not correlate with the degree of injury to the heart. The question of the predominant pathogenic role of exogenous or endogenous CA in this process remains unsolved.

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