PHARMACOLOGICAL INVESTIGATION OF THE MECHANISM OF THE RADIOPROTECTIVE EFFECT OF CATECHOLAMINES

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Experiments on albino mice irradiated in a dose of 685 R showed that the radioprotective effect of equimolar doses of adrenalin and isoprenaline (N-isopropylnoradrenalin) was identical (47% survival rate) and was greater than that of noradrenalin. Dibenamine slightly lowered the action of adrenalin and isoprenaline, but not of noradrenalin. Inetol (nethalide), alone or in combination with dibenamine, did not alter the radioprotective effect of the catecholamines. Anaprilin (propanolol) completely abolished the radioprotective action of isoprenaline but increased the effect of adrenalin (70% survival). The classical adrenergic receptors (α and β) evidently play no definite role in the radioprotective action of adrenalin and noradrenalin. Isoprenaline produces its effect through the β-receptors.

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The radioprotective action of the catecholamines has been known for many years [8, 9, 12], but few studies have been made of the receptors through which it is produced. It has been claimed that dibenamine reduces the radioprotective effect of adrenalin [13], but the factual evidence given in the paper is contradictory. Semenov [8] reports that "the most active adrenolytics (chlorpromazine, dihydroergotamine) sharply reduce the protective action of adrenalin," but these substances possess a highly complex action [3, 7], and they cannot be regarded as pure α-adrenolytics. On the other hand, evidence has been obtained of the prophylactic effect of dihydroergotamine [14]. The problem of the comparative effectiveness of adrenalin and noradrenalin likewise has not been definitely solved [13, 15]. Isoprenaline (N-isopropylnoradrenalin) also possesses radioprotective properties [6].

The object of this investigation was to determine the role of the classical adrenergic receptors (α and β) in the radioprotective action of the catecholamines by two methods: 1) by comparing the effects of noradrenalin (with high affinity for α-receptors), isoprenaline (stimulating mainly the β-receptors), and the ambivalent adrenalin; and 2) by studying the effect of the α-adrenolytic dibenamine and the β-adrenolytics inetol (nethalide) and anaprilin (propranolol), and combination of the two on the effectiveness of the catecholamines.

EXPERIMENTAL METHOD

Experiments were carried out on 1096 male albino mice of the same population aged 2-4 months and weighing 20-30 kg. The animals were kept on a balanced diet. Substances sysnthesized in the Khar'kov Institute of Endocrinology and Hormone Chemistry were used: L-adrenalin hydrotartrate 1 mg/kg, L-noradrenalin hydrotartrate 0.92 mg/kg (both given 15-20 min before irradiation), DL-isoprenaline hydrochloride 2.3 mg/kg 6-10 min before irradiation (doses of the catecholamines given are expressed as base, dibenamine 15 mg/kg 15-23 h before injection of the catecholamines, inetol 10 mg/kg 17-30 min before the catecholamines, and anaprilin 10 mg/kg, 25-30 min before the catecholamines. All the substances were made up before the experiment in 0.9% NaCl solution (dibenamine in distilled water) and injected subcutaneously in a volume of 8 ml/kg body weight. The animals were irradiated in a dose of 685 R (635 rad for soft tissues) under the following conditions: 190 kv, 15 mA, no filter, focal distance 15 cm, uniformity of distribution of dose over field within 9% limits, half-thickness layer 0.13 and 0.44 mm Cu. Survival rate after 30 days was determined. Differences between the series were assessed by the χ^2 method.

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TABLE 1. Survival Rate of Mice Irradiated in Dose of 685 R After Administration of Catecholamines, Adrenolytics, and Their Combinations

Adrenolytics	Physiological saline	Catecholamines		
		adrenalin	noradrenalin	isoprenaline
0.9% NaCl solution	1.3% (0.4-3.6) n=228 control	47%† (40-57) n=100	28%† (19-40) n=60	47%† (35-57) n=60
Dibenamine	8.0%* (2.6-16) n=50	29%† (19-41) n=55	30%† (18-44) n=40	30%† (19-43) n=46
Inetol (netalid)	12.0%† (4.5-22) n=40	45%† (26-65) n=20	30%† (14-58) n=10	45%† (26-65) n=20
Dibenamine + inetol	4.0% (0.2-17) n=25	45%† (26-65) n=20	33%† (14-58) n=15	45%† (26-65) n=20
Anaprilin (propanolol)	0% (0-26) n=10	65%† (44-82) n=20	—	6.5% (1.2-19) n=31
Dibenamine + anaprilin	0% (0-26) n=10	73%† (57-86) n=30	—	0% (0-28) n=9

Note. Confidence limits for survival rate given in parentheses (in %) obtained from a table of the binomial distribution for a coefficient of confidence of 0.95 [1]; n represents number of experiments.

*$P < 0.05$.

†$P < 0.001$ compared with control.

EXPERIMENTAL RESULTS

The mortality among the control mice was 99% (death occurred mainly on the 5th-12th day). All three catecholamines gave a well defined radioprotective effect, adrenalin and isoprenaline being equally effective in equimolecular doses (for the L-isomer), with a 47% survival rate, an effect 1.7 times better than that of noradrenalin (Table 1). When isoprenaline and noradrenalin were given together, 35 of the 60 mice (58%) survived, not significantly different from the radioprotective action of isoprenaline alone and less than the arithmetical sum of their effects (i.e., 75%).

Dibenamine itself had a slight prophylactic action (8%); it did not influence the effect of noradrenalin, but reduced the survival rate of the mice receiving adrenalin and isoprenaline.* In the dose used, dibenamine blocked the toxic action of the catecholamines. (The maximal tolerated dose of adrenalin for the irradiated mice was thus increased by not less than 10 times—up to 10 mg/kg.)

Inetol also led to the survival of some of the irradiated mice (12%), but it did not affect the radioprotective action of the catecholamines. A large dose of inetol (30 mg/kg) likewise did not change the effect of isoprenaline—5 of the 10 mice survived, compared with 1 of 10 mice after administration of inetol alone. A combination of dibenamine and inetol did not protect the animals and did not influence the radioprotective action of the catecholamines.

Anaprilin, whether alone or after preliminary administration of dibenamine, did not protect the mice and abolished the effect of isoprenaline almost completely, but the prophylactic action of adrenalin was actually increased. The radioprotective effectiveness of a combination of anaprilin and adrenalin (65-73% survival) was not inferior to that of cystamine (63% survival for n=79; 150 mg/kg intraperitoneally, 6-15 min before irradiation).

*When the significance of the action of adrenolytics on effectiveness of the catecholamines was assessed, a correction was introduced for the radioprotective action of the dibenamine and inetol themselves.

The results show that the pharmacological mechanisms of the radioprotective action of the catecholamines is complex. Inetol did not modify the protective action of isoprenaline, but this action was completely abolished by anaprilin, whose β-adrenolytic activity is from 10 to 20 times greater, in relation to some effects, than that of inetol [2, 10]. The radioprotective action of isoprenaline is evidently effected through the β-receptors. Neither inetol nor anaprilin depressed the prophylactic effect of adrenalin and, consequently, it is not connected with the β-receptors. The α-adrenolytic dibenamine did not abolish, but merely reduced (by about one-third) the radioprotective action of adrenalin and isoprenaline, and it did not modify the effect of noradrenalin in the dose used. Consequently, the α-receptors do not play the principal role in the protective action of sympathicomimetics which were studied. It also appeared improbable that the two types of receptors have a parallel action, for combinations of α- and β-adrenolytics did not reduce the radioprotective effectiveness of adrenalin and noradrenalin.

Whereas some sympathicomimetics give radioprotection through the β-receptors (isoprenaline; our own observations) and the α-receptors (phenylephrine; [15]), a leading role of the classical adrenergic receptors in the radioprotective action of catecholamines occurring in the body (adrenalin and noradrenalin) seems doubtful. It has been suggested that special and as yet unidentified receptors may exist. Evidence in support of this hypothesis is given by the fact that none of the adrenolytics used, nor any combination of them, completely abolished the radioprotective effect of adrenalin and noradrenalin. The higher effectiveness of adrenalin against the background of anaprilin can be explained on the assumption that blocking of the β-receptors increases the amount of adrenalin reacting with receptors of radioprotective action. This hypothesis naturally requires careful experimental verification.

The radioprotective effect of the catecholamines at the present time is explained by a decrease in the oxygen tension in the hematopoietic organs [4, 11, 13], possibly the result not so much of constriction of the blood vessels as of an increase in the oxygen utilization by the tissues [5]. The present investigation (specially the demonstration of a prophylactic effect of noradrenalin and isoprenaline, with their opposite actions on the lumen of the blood vessels) confirms observations that hemodynamic effects are not decisive in the radioprotective action of the catecholamines [8]. The use of adrenolytics seems very promising in the further study of this problem.

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