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Effect of cold stress on the subcellular distribution of noradrenaline in the rat heart

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Rats were exposed to cold (4°) for 2 hr to study the effect of increased sympathetic activities on the subcellular distribution of noradrenaline in the heart. Cold-exposure caused about 30% decrease of total noradrenaline contents in both auricles and ventricles of normal or adrenalectomized rats. This depletion of noradrenaline caused by cold was completely prevented by pretreatment of the rat with hexamethonium chloride. Measurement of the subcellular distribution of noradrenaline revealed that the percentage depletion in particle-bound amine in both auricles and ventricles was greater than in the supernatant noradrenaline. It is suggested that the noradrenaline in the particulate fraction is the functional pool of the amine available for release by nerve impulses.

SINCE the existence of two or more pools of noradrenaline stores in the sympathetically innervated organs was postulated by Trendelenburg (1961) and by others (see Kopin, 1964) there has been speculation about the functional pool of noradrenaline available for release by nerve impulses. Spontaneous junction potentials recorded in the vas deferens of the guinea-pig (Burnstock & Holman, 1962) indicate that in the adrenergic nerve, as in the cholinergic system, the neurotransmitter may be released in quanta. On the other hand, Euler & Lishajko (1962) suggested that noradrenaline in the free form might be the mobile pool, while Stjärne (1966) pointed out the impossibility of noradrenaline in granular vesicles being a functional pool on the basis of the number of the granular vesicles found in the sympathetic nerve.

Recently, Chang & Chang (1965) showed that when the isolated vas deferens of the rat was stimulated coaxially and then homogenized the decrease of noradrenaline was only found in the particulate fraction of the homogenized preparation. In contrast to this finding, Stitzel, Campos & Shideman (1965) reported that stimulation of the right accelerans nerve of the isolated perfused rabbit heart caused a decrease of the soluble noradrenaline fraction only.

It seemed, therefore, desirable to investigate the functional noradrenaline pool in intact animals and to attempt its localization. We have subjected rats to cold stress to increase the activity of the sympathetic innervation, and the effect of this treatment on subcellular distribution of noradrenaline in the heart has been examined. The results so far indicate that the content of noradrenaline in the particulate fraction decreased more than that in the supernatant fraction.

Methods

COLD STRESS

Long Evans rats of either sex, 250 to 300 g, were placed in a cold room at 4° for 2 hr after which the rats were killed by a blow on the head. The heart was then rapidly excised.

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SUBCELLULAR INVESTIGATION

The auricles, after being washed in cold saline, were homogenized in a loosely fitting glass homogenizer with 30 volumes of 0·25M sucrose in the cold for 2 min. Ventricles were homogenized with 10 volumes of 0·25M sucrose. A coarse fraction of the homogenates was removed by centrifugation at 1500 *g* for 5 min. This fraction usually contained about 40% of the total noradrenaline. The supernatant left after this initial centrifugation of the auricles (1 ml) was then layered on the surface of 0·4M sucrose (4 ml) and centrifuged at 39,000 rev/min (125,000 *g*) for 45 min in a Spinco Model L Ultracentrifuge with swinging bucket rotor SW 39. With this procedure, dilution of the auricle homogenate can be largely avoided. For the ventricles, aliquots (5 ml) of supernatant left after initial low speed centrifugation were centrifuged directly.

ASSAY OF NORADRENALINE

A modification of the trihydroxyindole method of Chang (1964) was used. The total content of noradrenaline in the auricle or ventricle was determined on 0·25 ml of the homogenate. For the assay of the noradrenaline in the supernatant from auricles, 1·5 ml of the topmost layer was used. High speed sediments were resuspended in 1 ml of 0·01N hydrochloric acid and assayed for particulate noradrenaline. Extraction of noradrenaline from each fraction was achieved by shaking with 10 volumes of acid-butanol to which excess of sodium chloride was added.

Results

EFFECT OF EXPOSURE TO COLD ON THE TOTAL NORADRENALINE CONTENT OF AURICLES AND VENTRICLES

Table 1 shows the effect of cold-exposure on the noradrenaline contents of auricles and ventricles. In agreement with the results of Leduc (1961),

TABLE 1. CHANGES OF THE TOTAL NORADRENALINE IN THE HEART OF RATS EXPOSED TO 4° FOR 2 HR (MEANS OF 6 EXPERIMENTS)

	Noradrenaline (μg/g ± s.e.)			
	Normal		Adrenalectomized	
	Auricle	Ventricle	Auricle	Ventricle
Control	3·54 ± 0·27	1·61 ± 0·21	4·36 ± 0·21	1·55 ± 0·12
Cold stressed	2·32 ± 0·25	1·11 ± 0·14	3·02 ± 0·25	0·97 ± 0·11
% Deviation from control	−34	−31	−33	−39
Statistical significance	P <0·02	P <0·1	P <0·01	P <0·01

cold exposure caused the noradrenaline of both auricle and ventricle to decrease by about 30%. Rats, which were adrenalectomized three days before the experiment and kept on normal saline, also showed a depletion of noradrenaline to a similar extent on exposure to cold (Table 1).

EFFECT OF GANGLIONIC BLOCKING AGENT

The decrease of the amine content of the rat heart in response to cold might be due to a decreased rate of synthesis, or to impairment of the storage mechanism, induced by a lowering of the body temperature. Hexamethonium chloride (10 mg/kg) was given to rats subcutaneously 20 min

before cold-exposure. Table 2 shows that treatment with this ganglionic blocking agent completely protected the amine both in auricles and ventricles from being decreased in response to cold. This would indicate

TABLE 2. EFFECT OF HEXAMETHONIUM ON THE CHANGE OF NORADRENALINE INDUCED BY COLD STRESS
Hexamethonium chloride (10 mg/kg) was given to rats subcutaneously 20 min before exposure to cold (means of six experiments)

	Noradrenaline (μg/g ± s.e.)	
	Auricle	Ventricle
Control	3·76 ± 0·25	1·71 ± 0·18
Cold stressed	3·58 ± 0·55	1·70 ± 0·30
Statistical significance	P >0·4	P >0·5

that the loss of noradrenaline in the animals not treated with hexamethonium (Table 1) may be mediated through the increased sympathetic nerve activities.

CHANGE IN THE SUBCELLULAR DISTRIBUTION OF NORADRENALINE

Measurement of noradrenaline in the subcellular fractions of the auricles from cold-stressed animals revealed that the particulate fraction lost more than 50% of its amine, while supernatant lost only 25% (Table 3).

TABLE 3. CHANGES IN THE SUBCELLULAR DISTRIBUTION OF NORADRENALINE IN RESPONSE TO COLD (MEANS OF 6 EXPERIMENTS)

	Noradrenaline (μg/g ± s.e.)					
	Auricle			Ventricle		
	Supernatant (S)	Particulate (P)	Ratio (P/S)	Supernatant (S)	Particulate (P)	Ratio (P/S)
Control ..	0·96 ± 0·04	0·58 ± 0·05	0·61 ± 0·06	0·37 ± 0·04	0·36 ± 0·04	0·99 ± 0·05
Cold stressed ..	0·72 ± 0·11	0·24 ± 0·04	0·37 ± 0·07	0·26 ± 0·03	0·20 ± 0·03	0·82 ± 0·09
% Deviation from control	−25	−59		−30	−44	
Statistical significance ..	0·05 < P <0·1	P <0·01	P <0·05	P <0·05	P <0·01	P = 0·1

The ratio of the amount of noradrenaline in the particulate fraction to that in the supernatant was thus reduced from 0·61 to 0·36 (P <0·05). The loss of particulate noradrenaline of ventricles also exceeded that of supernatant amine (44% as against 30%) although the difference is not as marked as in the auricles and is statistically not significant.

Discussion

Hsieh, Carlson & Gray (1957) pointed out the importance of noradrenaline released from the sympathetic nerve endings for the generation of heat other than by shivering. The circulatory level of noradrenaline in the cat exposed to cold was increased (Hemingway & Price, 1964) and urinary excretion of the amine in the rat exposed to 3° was also elevated (Leduc, 1961). The view that noradrenaline released in response to cold comes largely from sympathetic nerves is supported by evidence that, in adrenalectomized animals, the urinary excretion of noradrenaline still

increases to the same extent, so the response is not a secondary effect of adrenaline release from the adrenal medulla which might displace noradrenaline in the heart (Angelakos, Bloomquist & King, 1965), and also that a ganglionic blocking agent inhibits the increase of urinary noradrenaline excretion (Leduc, 1961). It was further shown by Leduc that the noradrenaline content in the rat organs innervated by sympathetic nerves decreased on exposure to cold, whereas Johnson (1964) reported a negative result.

In the present experiments we have confirmed the finding of Leduc that noradrenaline in the heart decreases in response to cold. Since this decrease of noradrenaline content was prevented by pretreatment of the animal with hexamethonium, it is unlikely that the decrease of tissue noradrenaline after exposure to cold is due to an impaired rate of noradrenaline synthesis caused by the fall in body temperature, to an impaired storage mechanism, or to increased metabolic turnover. If it were a temperature effect, hexamethonium treatment, which prevents the calorigenic effect in response to cold (Hsieh & others, 1957; Gilgen, Maickel, Nikodijevic & Brodie, 1962; Brück & Wünnenberg, 1965), should on the contrary enhance the decrement of noradrenaline. It can be concluded therefore that the decrease of heart noradrenaline in the rat exposed to cold is the result of increased release of noradrenaline from sympathetic nerve endings due to increased nervous activity.

The data in the present experiments show that, in the auricle, noradrenaline in the particulate fraction decreased more than that in the supernatant fraction on exposure of the rat to cold, suggesting that the particulate noradrenaline rather than the supernatant amine may be immediately involved in sympathetic nerve transmission. Although cold stress did not significantly change the pattern of subcellular distribution of noradrenaline in the ventricles, the result shows a similar tendency to depletion. It might be that the size of the functional pool of the transmitter amine in the ventricles is smaller than that in the auricle. The result obtained with the auricles agrees with that obtained by coaxial stimulation of the isolated vasa deferentia of rats (Chang & Chang, 1965), but is different from that obtained in the isolated rabbit heart (Stitzel & others, 1965). By stimulation of the right accelerans nerve of the isolated perfused rabbit heart for 5 to 10 min, Stitzel & others (1965) found a decrease of soluble noradrenaline in both auricle and left ventricle while no significant change was found in the particulate fraction. However, in their experiment, stimulation for more than 10 min did not increase the effect further. The results of Iversen, Glowinski & Axelrod (1965) showed that the ratio of particulate to supernatant [^{3}H]noradrenaline of the isolated perfused heart was markedly different from the same ratio of the heart, *in situ* (0·6 and 1·5 respectively). This might suggest that in the isolated perfused heart the ability of 'particles' to hold noradrenaline is impaired and such an existing impairement of storage or release mechanism in this preparation must be taken into consideration. It is interesting that Hift & Campos (1962) also found a greater decrease of noradrenaline from the particulate fraction in dogs under irreversible

haemorrhagic shock, a condition in which sympathetic discharge is greatly increased (Millar, Keener & Benfey, 1959; Neil, 1962). It appears that sympathetic activities induced either by cold-exposure or by haemorrhagic hypotension tend to deplete particulate noradrenaline more than the supernatant noradrenaline from the heart. The soluble fraction of noradrenaline is therefore unlikely to be the functional pool on which nerve impulses would directly act, as suggested by Euler & Lishajko (1962) and Stitzel & others (1965).

The electrophysiological finding on vas deferens by Burnstock & Holman (1962), of the quantal nature of the spontaneous junction potentials, strongly indicates that the transmitter released from the sympathetic nerve is contained in packets as in other systems. In studies on the particulate binding of noradrenaline and related compounds Musacchio, Fischer & Kopin (1966) found that only those sympathomimetic amines, which are retained in noradrenaline storage vesicles, can be released by sympathetic nerve stimulation. They suggested that the vesicles or a binding mechanism with similar properties is the site from which noradrenaline is released when impulses reach the nerve endings. Our results, which show that increased sympathetic activities in the rat heart induced by exposure to cold primarily release noradrenaline from the particulate fraction, also point to the possibility of particle-bound noradrenaline as the functional pool available for release by nerve impulses. The same conclusion has been reached from experiments using the isolated vas deferens preparation of the rat (Chang & Chang, 1965).

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