Close relation between hypothalamic and cardiac norepinephrine during stress and its role in acute myocardial infarction disrhythmias

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Summary. The decrease of the norepinephrine levels in hypothalamus and heart caused by stress is prevented by pargyline and imipramine. Such a decrease in spleen and adrenals is not affected. Chlorpromazine and lithium only prevent the norepinephrine decrease in the spleen. The uptake of H^3norepinephrine by isolated atria of guinea-pig increases during anoxia; the change to a normal oxygen situation decreases these norepinephrine levels by more than 50%.

Several forms of experimental stress result in decreased brain norepinephrine(NE)[1-5]. According to O'Boyle et al.[6], many of the symptoms of human myocardial infarction (which is a stress condition) are related to increased catecholamine release. The relevance of the NE locally released by sympathetic terminals, independently of the NE released by the adrenals, is also well documented in stress conditions[7].

Ordy et al.[8] found (in monkeys) a significant correlation between the amount in NE present in the hypothalamus and that in the heart after treatment with anaesthetic and tranquillizer (chlorpromazine and haloperidol) drugs, and after experimental stress and its reversal. Previous studies from our laboratory show that, in rats, the decrease in hypothalamus, heart and spleen NE induced by immobilization stress is prevented by pretreatment with pargyline.

All these facts led us to think that the alterations in cardiac adrenergic neurons caused by stress could be dependent on changes in hypothalamic adrenergic neurons. If this hypothesis could be confirmed, it would be feasible to envisage a new, more aethiological therapy for the cardiac sympathetic hyperactivity present during stress, by means of drugs whose target was the hypothalamus rather than the heart.

Methods. Determination of endogenous NE. Male Sprague Dawley rats (300 g b.wt) were used. Chlorpromazine (10 mg/kg), lithium chloride (100 mg/kg), pargyline (100 mg/kg) and imipramine (100 mg/kg) were injected i.p. for 3 days. 1 group of animals were immobilized for 24 h. Immobilized and control rats were killed by decapitation. Hypothalamus, spleen, heart and adrenal were rapidly blotted, weighed and homogenized in 5 ml of ice-cold 0.4 N perchloric acid. Purification of endogenous NE on Al_2O_3 was carried out by the method of Anton and Sayre[9]. The endogenous NE was assayed by the spectrofluorometric technique[9]. All data were corrected for recovery and expressed as percent of their corresponding control values.

H^3NE uptake and retention. Guinea-pigs weighing approximately 500 g were used. The animals were killed by a blow on the head, the hearts were quickly removed and the left atria were prepared as described by Furchgott and Sanchez-Garcia[10]. The incubation medium was Krebs-bicarbonate solution with ethylene diamine tetraacetic acid. The temperature was kept constant at 37 °C. In a single experiment, one half served as the control and the other half as the experimental preparation. Anoxia was produced by 95% N_2 - 5% CO_2. This gas mixture was then continuously bubbled through the control muscle chamber. During anoxia, both atrial halves were then incubated with 10 µCi of d,lH^3NE for 5 min and then thoroughly washed. After incubation, experimental half was then bubbled with 95% O_2 - 5% CO_2 for 30, 60 and 120 min. Atria were blotted on a filter paper. Purification of H^3NE was carried out by method described above. Radioactivity was determined in a liquid scintillation counter (Nuclear Chicago 725) using the scintillation solution of Bray[10]. Quenching correction was determined by the channel ratio method using a quench calibration curve. Radioactivity is expressed as disintegrations per min/g of wet tissue. Differences between means were assessed by Student's t-test. Values given are means ±SE.

Results. 1. Effects of drug pretreatment on the changes induced by immobilization stress in the levels of endogenous NE. They are shown in figure 1. Immobilization stress decreases the levels of NE in hypothalamus ($p < 0.05$), heart ($p < 0.01$), adrenals ($p < 0.001$) and less clearly in spleen ($p < 0.1$). Pretreatment with imipramine prevents that NE decrease in hypothalamus and heart induced by stress. Pargyline does so in hypothalamus, heart and spleen. Chlorpromazine and lithium only prevent the NE decrease in spleen. None of the 4 drugs used prevented the depletion caused by stress in adrenals.

2. Effects of anoxia on the uptake and retention of H^3NE in isolated atrium of guinea-pig. As figure 2 shows, both the uptake and retention of H^3NE under anoxia decreased significantly after O_2 administration ($p < 0.01$ after 1 h of O_2 and $p < 0.001$ after 2 h).

Discussion. Our experiments show how the stress-induced NE depletion in hypothalamus and heart can be prevented by pretreatment with pargyline and imipramine, in contrast with the effects of chlorpromazine and lithium chloride.

There is clear parallelism between the hypothalamic and cardiac responses to all 4 drugs studied. There is no parallelism, however, between the hypothalamic and cardiac responses and the responses of the adrenals or the spleen. This suggests that the effects on the heart are mainly not local in nature, in that case the effects on the adrenals and the spleen would presumably have been in the same direction as the effect on the heart. It points rather to the conception that the cardiac responses are extracardiac in origin, and conceivably related to the hypothalamic responses.

There is some discrepancy between our chlorpromazine results and those published in available reports. According to Ordy et al.[8], 15 mg/kg of chlorpromazine prevent the NE depletion produced in hypothalamus and heart of monkeys by electric shock. The same effect is produced by 10 mg/kg on the depletion induced by electric shock in the brain stem of rats according to Maynert[1]. These discrepancies could be accounted for in terms of differences in the doses and species used[8], and in the stress situation[1,8] and the areas of the brain considered[1]. In our hands, chlorpromazine 10 mg/kg only prevented the stress-induced NE depletion in the spleen, which is the organ in which the depletion is less consistent, and presumably even a very weak local action might be sufficient to prevent it. The local action of our dose of chlorpromazine, however, was not enough to produce changes in the cardiac NE depletion.

A logical explanation of the results obtained by using pargyline would be to assume that the NE released by stress is prevented from deamination by MAOI. In these terms, the reversal effect by pargyline would be but a normal consequence of inhibition of deamination with no signifi-

cant functional relevance. However, the application of tyramine to an isolated atria of a guinea-pig (pretreated with pargyline) significantly reduced levels of NE (non-published data). In this case it would have been expected that the stress (similarly to those stimuli resulting from tyramine treatment) would reduce NE levels of stressed animals under pargyline treatment. On the other hand, if reversal effects of pargyline could be just explained in terms of its inhibition effects on MAO, no significant decline in the amount of NE in adrenals would have appeared.

It is well known that MAO inhibitor drugs cause ganglionic blockade by increasing the levels of NE in ganglia as they decrease its deamination[11-15]. This circumstance would account for our results with pargyline on the spleen and the heart; the fact that pargyline does not protect the adrenals from stress-induced NE depletion, does not, however, support such a possibility. The simplest explanation would be a local action on the spleen and heart themselves. Pretreatment with pargyline (unpublished observations) greatly increases the NE levels in all brain areas, and in the spleen and heart, both in stressed and control rats. The results reported here do not exclude a local action of MAOI drugs on the spleen and heart. They do not exclude either the possibility that, apart from such an action, they could exert a reflex one with hypothalamic participation.

O'Boyle et al.[6] found an increase in the urinary excretion of catecholamines and its metabolites during acute myocardial infarction, and thought it relevant to the production of existing cardiac disrhythmias, both supraventricular and ventricular. According to Mendez (personal communication), the membrane alterations present during postinfarction disrhythmias can reflect 2 circumstances: hypoxia and increase in beta adrenergic receptor activity. It has been shown that guinea-pig isolated atria increase their uptake and retention of H^3NE during hypoxia[17]. In a previous report, we presented evidence in favour of the idea of the increase in H^3NE uptake resulting from a decreased capacity of monoaminooxidases to cause oxidative deamination in the absence of oxygen. The results reported here show a significant increase in the uptake and retention of H^3NE by isolated guinea-pig atria during anoxia, with subsequent recovering after restoring oxygen. This supports the assumption of oxygen playing an important role in those phenomena. Additional support for this hypothesis comes from the fact that the increase in H^3NE uptake and retention during anoxia is even bigger in guinea-pig isolated atria from reserpinized animals pretreated with MAOI drugs[19]. The reversibility of the effect of anoxia on MAO activity after restoring the oxygen is similar to the reversibility (of the MAO inhibition) caused by tyramine in guinea-pig isolated atria pretreated with iproniazide[20].

As a recapitulation of the various facts discussed so far, it is possible to make some suggestions: A) The increase in NE levels during acute myocardial infarction could be due to MAO inhibition caused by hypoxia. B) Simultaneous nervous hyperactivity due to stress could, very likely, result in a conspicuous increase in the release of NE, not only from the NE physiologically available stores but also from the increased supplies available during anoxia. C) The subsequent beta receptor hyperactivity could well play a causal role in the disrhythmias of acute myocardial infarction.

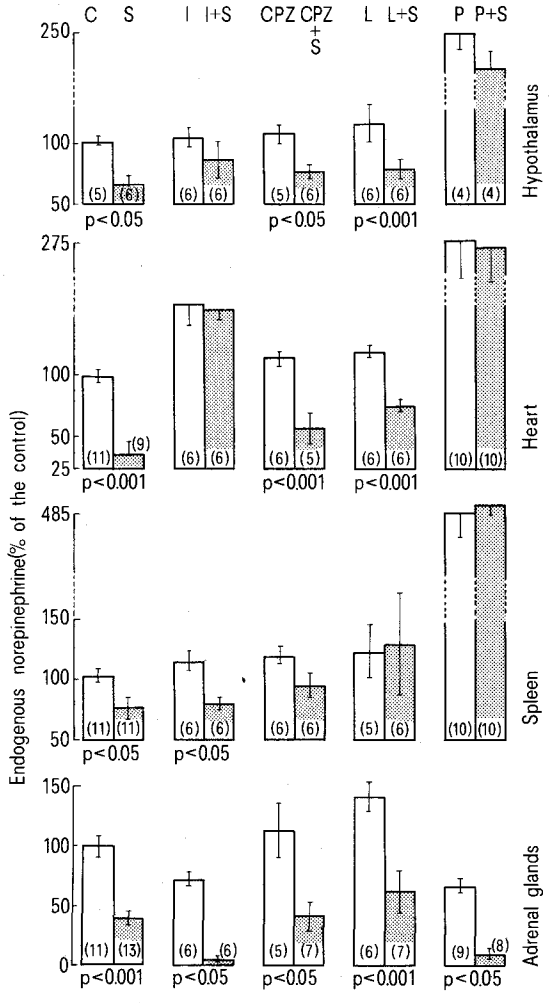

Fig. 1. Endogenous norepinephrine and pretreatment with drugs. Absolute control levels of NE (μg/g of tissue): hypothalamus, 1.735±0.075; heart, 0.639±0.074; spleen, 0.835±0.077; adrenal glands, 957.806±65.700. C, Controls, no stress; S, animals under immobilization stress; I, CPZ, L, P, imipramine-, chlorpromazine-, lithium chloride- and pargyline-treated controls; I+S, CPZ+S, L+S, P+S, stressed animals pretreated with imipramine, chlorpromazine, lithium chloride and pargyline. The number of animals studied in parentheses. Vertical bars indicate SEM.

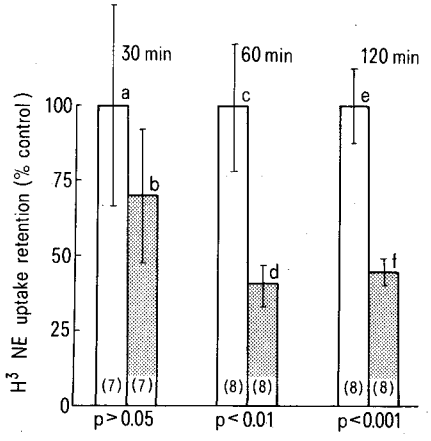

Fig. 2. H^3Norepinephrine uptake and retention by guinea-pig isolated atrium during anoxia and the change to a normal oxygen situation. a, c, e, Controls; after H^3NE incubation during anoxia, this gas mixture was then continuously bubbled for 30, 60 and 120 min. b, d, f, After H^3NE incubation during anoxia, oxygen was bubbled for 30, 60 and 120 min. Vertical bars indicate SEM. The number of experiments in parentheses.

D) It is conceivable that the close relation between hypothalamic and cardiac NE during stress could be relevant for the clinical management of some situations, including acute myocardial infarction.

1 E.W. Maynert and R. Levi, J. Pharmac. exp. Ther. *143*, 90 (1964).
2 E.L. Bliss, J. Ailion and J. Zwanziger, J. Pharmac. exp. Ther. *164*, 122 (1968).
3 L.A. Carr and K.E. Moore, Neuroendocrinology *3*, 285 (1968).
4 E.A. Stone, J. Neurochem. *21*, 589 (1973).
5 M. Palkovits, R.M. Kovayashi, J.S. Kizer, D.M. Jacowitz and I.J. Kopin, Neuroendocrinology *18*, 144 (1975).
6 A. O'Boyle, D. Gannon and D. Hingerty, J. Irish med. Ass. *66*, 699 (1973).
7 R.J. Wurtman, New. Engl. J. Med. *273*, 637 (1965).
8 J.M. Ordy, T. Samorajski and D. Schroeder, J. Pharmac. exp. Ther. *152*, 445 (1966).
9 A.H. Anton and D.F. Sayre, J. Pharmac. exp. Ther. *138*, 360 (1962).
10 R.F. Furchgott and P. Sanchez-Garcia, J. Pharmac. exp. Ther. *163*, 98 (1968).
11 G.A. Bray, Analyt. Biochem. *1*, 279 (1960).
12 L. Guillespie, L. Terry and A. Sjoerdsma, Am. Heart J. *58*, 1 (1959).
13 S.B. Gertner, Nature *183*, 750 (1959).
14 S.B. Gertner, J. Pharmac. exp. Ther. *131*, 223 (1960).
15 L.I. Goldberg and F. da Costa, Proc. Soc. exp. Biol. Med. *105*, 223 (1960).
16 M. Puig, A.R. Wakade and S.M. Kirpekar, J. Pharmac. exp. Ther. *182*, 130 (1972).
17 A.R. Wakade and R.F. Furchgott, J. Pharmac. exp. Ther. *163*, 123 (1968).
18 R. Martinez-Sierra, F. Andres-Trelles, C. Vazquez-Herrero and P.D. Garcia de Jalon, Proc. 6th int. Congr. Pharmac. Abstr. 245, Sanomaprint, Helsinki 1975.
19 R. Martinez-Sierra, A. Velasco-Martin and P.D. Garcia de Jalon, Experientia *29*, 568 (1973).
20 P.D. Garcia de Jalon, R. Martinez-Sierra and A. Velasco-Martin, Br. J. Pharmac. *48*, 609 (1973).
21 A.M. Thierry, G. Blanc and J. Glowinski, Eur. J. Pharmac. *10*, 139 (1970).

Sodium excretion after bilateral adrenalectomy in rats with experimental cirrhosis

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Summary. Bilateral adrenalectomy (ADX) or a sham procedure was performed in cirrhotic and control rats. After ADX, controls increased their sodium excretion with respect to the basal values but cirrhotics did not. When sodium-loaded, the ADX cirrhotic rats retained a significant amount of the load. These data do not support a primary role of aldosterone in the impaired sodium handling by cirrhotic rats.

An increase of aldosterone plasma levels has been claimed to have a primary role in water and salt retention by the kidney in chronic hepatic disease[2,3] and other situations which generate oedema and ascites[4]. Marson[5] observed natriuresis after bilateral adrenalectomy (ADX) in a patient with hepatic cirrhosis who had not responded to habitual therapeutics. Disappearance of oedema and improvement of sodium excretion were also observed[6,7]. To explore the problem, sodium excretion was followed after bilateral ADX on rats in which chronic experimental cirrhosis had been produced. The study was carried out in male Wistar rats. Experimental cirrhosis was induced in a group of 17 rats weighing about 150 g by a combinated treatment of sodium phenobarbital (Luminal®, Bayer) given orally and Carbon tetrachloride by inhalation, according to the schedule reported by Lopez-Novoa et al.[8,9]. All the animals had, at the time of the experiment, histologically proven cirrhosis and showed a variable amount of ascites. Their weight averaged 249±3 g (SEM). 16 rats drinking sodium phenobarbital chronically were used as controls to obviate the possible effects of the Luminal administration on sodium handling. The control rats did not show any histological alteration in the liver and their weight was not different from that of cirrhotic rats (254±3 g, $p > 0.01$).

All the animals were placed into individual metabolic cages with free access to drink and a fixed amount of standard rat food in powdered form (total sodium content 0.605±0.091 mEq; 5 determinations). The animals remained in the metabolic cages for 5 days. On the 6th day, after light ether anesthesia, a bilateral ADX was performed in 11 cirrhotic and 10 control rats, by dorsal incision. 6 cirrhotic and 6 control rats were subjected to the same procedure but adrenalectomy was not performed. Strict care was taken to prevent loss of ascitic fluid. The animals were again placed into the metabolic cages for 3 days. Afterwards, the drink was substituted by 30 ml of a sodium chloride solution,

Sodium excretion after adrenalectomy (ADX) and Na load

		Cumulative Na excretion		Cumulative Na balance		Percentage of the Na load excreted
		Prior ADX	After ADX	Prior ADX	After ADX	
Control, ADX	$\bar{x}$	1.78	3.71[c]	+0.02	−1.90[c]	84.9
(n=10)	SEM	0.11	0.35	0.06	0.16	3.2
Control, sham	$\bar{x}$	1.76	1.67[a]	+0.04	+0.12	86.2
(n=6)	SEM	0.10	0.19	0.09	0.09	4.3
Cirrhosis, ADX	$\bar{x}$	1.24[a]	1.56[a]	+0.56[a]	+0.23[a]	64.3[a]
(n=11)	SEM	0.07	0.17	0.06	0.13	4.8
Cirrhosis, sham	$\bar{x}$	1.26[a]	1.32[a]	+0.54[a]	+0.48[a]	41.4[a b]
(n=6)	SEM	0.17	0.14	0.15	0.13	4.4

All the data are expressed as mEq in 3-day periods. [a] $p < 0.05$ with regard to the group control-ADX; [b] $p < 0.05$ with regard to the group cirrhosis-ADX; [c] $p < 0.05$ with regard to the period previous to ADX.