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Correlation between Increased Dopamine-β- Hydroxylase Activity and Catecholamine Concentration in Plasma: Determination of Acute Changes in Sympathetic Activity in Man ***

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Summary. In 11 healthy untrained volunteers the increase in plasma dopamine-β-hydroxylase (DBH) activity during graded physical exercise has been examined as a true measure of increased activity of the sympathetic nervous system. The correlation between DBH activity, catecholamine concentration (CA) in plasma and heart rate was studied. When work on an electrically braked bicycle ergometer was gradually increased from 12.5 to 100, 200 and 300 watts there was a linear increase in DBH activity and heart rate; the increase in CA concentrations followed an exponential function. The peak values for DBH activity and CA concentration in plasma after the 300 watt work load (as percentages of the resting levels) were 130±3% and 820±71%, respectively; the adrenaline concentration in plasma increased only to 150±19% ($p > 0.05$). There were significant correlations between heart rate and work load, DBH and work load and log CA and work load. The data imply direct correlations between heart rate and DBH, heart rate and log CA and DBH and log CA. The exponential increase in noradrenaline concentration in plasma might be due either to a greater net "overflow" from sympathetic nerve endings, and/or to increased secretion by the adrenal medulla. In the latter case, the release of noradrenaline would not be accompanied by secretion either of adrenaline or DBH. After work ceased there were sharp falls in heart rate and CA concentration, which indicate an immediate drop in sympathetic activity. DBH activity in plasma returned to normal very slowly; it reached half maximum values after 20 - 22 min. It is concluded that increased sympathetic activity in man can be estimated in vivo as changes in DBH and/or CA concentration in plasma. In contrast, a rapid decrease in sympathetic activity is directly reflected only by a rapid fall in the plasma concentrations of CA.

Key words: Sympathetic activity, plasma catecholamine concentration, dopamine-β-hydroxylase activity, graded physical exercise, heart rate, man.

Previous investigations have shown that acute changes of sympathetic activity in man may be reflected by changes in dopamine-β-hydroxylase (DBH) activity in plasma (Planz and Palm, 1973; Planz *et al.*, 1973; Wooten and Cardon, 1973; Wiethold *et al.*, 1973). Stimulation of sympathetic nerves releases the enzyme DBH from their endings, where it is stored together with noradrenaline (reviewed by Molinoff and Axelrod, 1971; Axelrod, 1972; Goldstein *et al.*, 1972). According to the mechanism of exocytosis underlying the release of the adrenergic transmitters noradrenaline and adrenaline from storage granules of sympathetic nerve terminals and chromaffin cells, it is postulated that catecholamines (CA) and DBH should be released in a constant stoechiometric relation into the extraneuronal space (for review see Axelrod, 1972). This has been confirmed in vitro by electrical stimulation of sympathetically innervated organs (Smith *et al.*, 1970; De Potter *et al.*, 1970; Weinshilboum *et al.*, 1971b; Wooten *et al.*, 1973). Elevation of DBH activity in plasma has been demonstrated in animals (chronic experiments) during enhanced sympathetic activity under various experimental conditions as well as in acute studies in man (Weinshilboum *et al.*, 1971a, Planz and Palm,

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1973; Roffman *et al.*, 1973; Wooten and Cardon, 1973).

Comparisons have not yet been made, however, between increased DBH activity and elevated CA concentrations in plasma. It appeared of value, therefore, to investigate any possible relationship between these two parameters in man during stimulation of sympathetic activity by gradually increasing physical exercise. Such a correlation would mean that changes in DBH activity would reflect alterations in sympathetic activity.

Methods and Materials

The investigations were performed in 11 healthy, untrained volunteers, with a mean age of 29 ± 1.8 years. No drugs were allowed 24 hours before each experiment. After resting for 30 minutes in the supine position, graded physical exercise was performed while sitting on an electrically braked bicycle ergometer. During the exercise period of about 12 minutes, the work load was gradually increased from 12.5 watts (5 min) to 100 and 200 watts (each for 3 min), and to 300 watts (1 min; Fig. 1). Work load was elevated to the next step when the heart rate, recorded continously from the ECG, had reached a higher and almost constant level. Under these experimental conditions almost steady state conditions were achieved. Thus, during a constant work load of 100 watts over a period of 20 minutes, heart rate, plasma catecholamine (CA) concentration, and plasma DBH activity, remained almost constant after a rapid initial rise (CA concentration + 110 ± 10%; DBH activity + 9 ± 1%; heart rate + 60 ± 6%). Just before each increase in work load, blood samples of about 25 ml were drawn from a cubital vein through a venous catheter, and after heparinisation the blood was kept in ice. After termination of the work load the volunteers again lay supine and blood samples were collected 5, 15 and 30 minutes after the end of the exercise.

CA concentration in plasma was measured fluorimetrically in samples equivalent to 10 ml plasma according to the methods of Anton and Sayre (1962), and Renzini *et al.*, (1970). Deproteinized plasma samples were adsorbed on Al_2O_3 (Woelm, Eschwege; activity grade III) and eluted with 1.5 ml 0.6 M boric acid in 2 N HCl (20 : 1 v/v). 1 ml was used for the fluorimetric procedure according to Renzini *et al.* (1970). In order to differentiate between noradrenaline and adrenaline, i.e. between the parameters of sympathetic and sympatho-adrenal activity, both CA were determined in 0.5 ml plasma samples according to the method of Passon and Peuler (1973); S-adenosylmethionine-methyl-^{3}H (spec. activity 8.4 Ci/mmole; New England Nuclear Corporation, Boston) was used as the donor of methyl groups for methylation of CA. The values for CA given in text and figures were not corrected for recovery.

DBH activity in plasma was determined radiometrically after Weinshilboum and Axelrod (1971a), using tyramine as the substrate, as described previously (Planz and Palm, 1973). Phenylethanolamine-N-methyltransferase was purchased from Miles Laboratories, Slough, England; S-adenosylmethionine-methyl-^{14}C (spec. act. 58 mCi/mmole) was obtained from the New England Nuclear Corporation, Boston.

Resting values of plasma DBH activity in the 11 volunteers ranged from 66 to 492 units ($\bar{x}$ ± SEM = 208 ± 39 units). Because of the wide range of individual variations (c.f. Axelrod, 1972), changes in enzyme activity were expressed as percentages of the respective individual resting value (= 100%. For details, see 'Discussion').

Results in the text and figures have been expressed as mean values ± SEM from n individual experiments. Statistical differences were examined by R.A. Fisher's t-test. The linearity of the regression functions were tested by the F-distribution.

For the statistical evaluations we are deeply indebted to Dipl.-Math. H. Decker; c/o E.Merck, Darmstadt.

Results

*DBH and catecholamine (CA) co*ncentration in plasma and heart rate measured simultaneously increased greatly during graded physical exercise that increased stepwise from 12.5 to 100, 200 and 300 watts. After the highest work load (300 watts for about 1 minute) the peak values of heart rate, CA concentration and DBH activity were 272 ± 15%, 820 ± 71%, and 130 ± 3%, respectively. As shown in Fig. 1, heart rate and CA concentration increased almost in parallel depending on the work load. When exercise ceased, both heart rate and CA concentration decreased rapidly and reached about 30 per cent of their maximum values within 5 minutes.

Under the same conditions, plasma DBH showed no significant increase at the lowest work load, even though heart rate and CA concentration had already risen. Rapid elevation of enzyme activity occurred at higher work loads, i.e. at 100, 200 and 300 watts, almost in parallel with the changes in heart rate and CA concentration.

Compared to the rapid decrease of heart rate and CA concentration after termination of exercise, the decline in DBH activity was much slower, and half maximum activity was only reached at 22 minutes. After one hour, DBH was in the range of the initial resting values (not shown in the figure). The changes in DBH, CA concentration and heart rate at the various work loads (expressed as absolute values with the exception of DBH) are depicted in Fig. 2. Heart rate and DBH activity increased almost linearly, whereas plasma CA concentration showed more pronounced elevation at higher work loads compared to those found at lower work loads.

From Fig. 3, it is apparent, that CA in plasma increased logarithmically depending on the various work load steps, whereas the increases in heart rate and DBH were directly related to the increased work load. In all three cases, the test quotient of the F-distribution did not attain the limit of significance (P = 0.05; c.f. data in Figs. 2 and 3).

From this, it can be assumed that the increases in heart rate and DBH activity were directly related (Fig. 4). There was also a correlation between the increase in heart rate and the logarithms of the corresponding CA concentrations, which may be considered a direct relationship (Fig 5) according to the data in Figs. 2 and 3. Finally, the results show a significant correlation between enhanced plasma CA concentration and plasma DBH activity during physical exercise: a direct correlation between DBH activity and the logarithm of CA can be assumed (Fig. 6), based on the results and calculations depicted in Figs. 2 - 5.

Discussion

The results obtained in man during physical exercise have demonstrated a simultaneous increase in plasma DBH activity and plasma CA concentration; both parameters were dependent on the magnitude of the work load. This correlation suggests that these biochemical parameters in plasma reflect acute changes in sympathetic activity. Noradrenaline and DBH are known to be stored in the same compartment, i.e. in the storage vesicles of sympathetic nerve endings; both components are released in a constant relation in response to sympathetic nerve stimulation (for review, see Axelrod, 1972). These experiments in man provide the first experimental proof of simultaneous release of both components during activation of the sympathetic nervous system. They are in accordance with results obtained by in vitro experiments (Smith *et al.*, 1970; De Potter *et al.*, 1971; Johnson *et al.*, 1971; De Potter *et al.*, 1972; Wooten *et al.*, 1973).

Several limitations, however, must be kept in mind: noradrenaline released from the sympathetic nerve endings into the synaptic cleft is rapidly inactivated enzymatically and by a highly efficient re-uptake mechanism; after diffusion into the intravasal space it is rapidly eliminated from the circulation by the kidneys. On the other hand, no information is yet available about how DBH (mol wt 290 000; c.f. Axelrod, 1972; Wallace *et al.*, 1973) gains access to the circulation after release from the nerve terminals. The mechanism involved in DBH inactivation, before or after reaching the circulation, is also unknown. Furthermore, during physical stress CA and DBH may also be released from the adrenal medulla. Thus, changes in CA and enzyme activity should reflect changes in the entire sympatho-adrenal system. Keeping in mind these putative limitations, the present experimental data can be interpreted as follows:

1. Although a significant increase of CA concentration in plasma was found after a work load of 12.5 watts for 5 min, there was virtually no change of DBH activity (c.f. Fig. 1, 2). This may be explained by rapid diffusion of CA from the synaptic cleft into the blood stream after release from sympathetic nerves, whereas the diffusion of DBH may be much slower, and probably follows its partial inactivation in the interstitial space. This assumption is supported by the results of Freedmann *et al.* (1973), who found, after a brief cold pressure test, that enhancement of DBH *concentration* in plasma (determined by an immunologi-

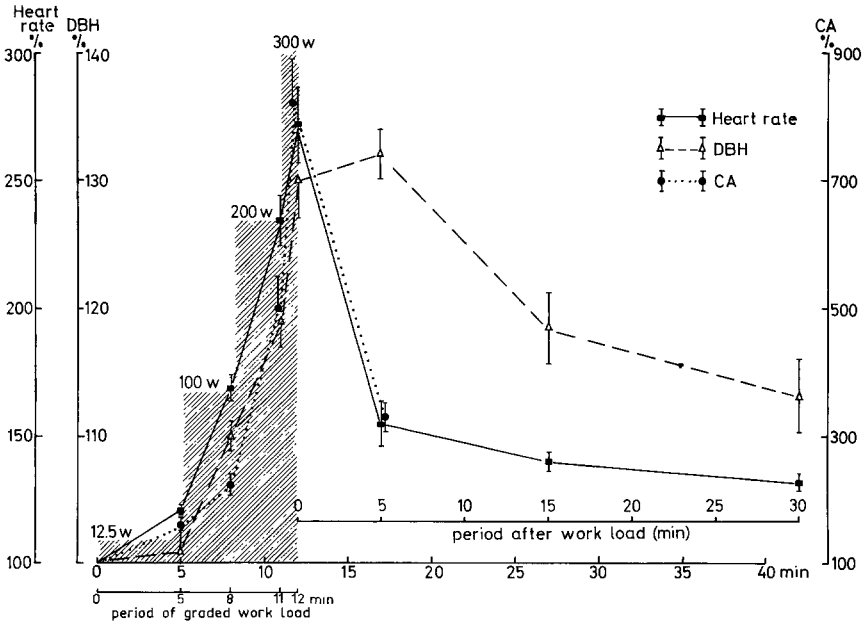

Fig. 1. Changes in heart rate, dopamine-β-hydroxylase activity (DBH) and catecholamine concentration (CA) in human plasma during and after graded physical exercise on a bicycle ergometer (W=watts). Abscissa: time in minutes (min); ordinates: values in % of resting values

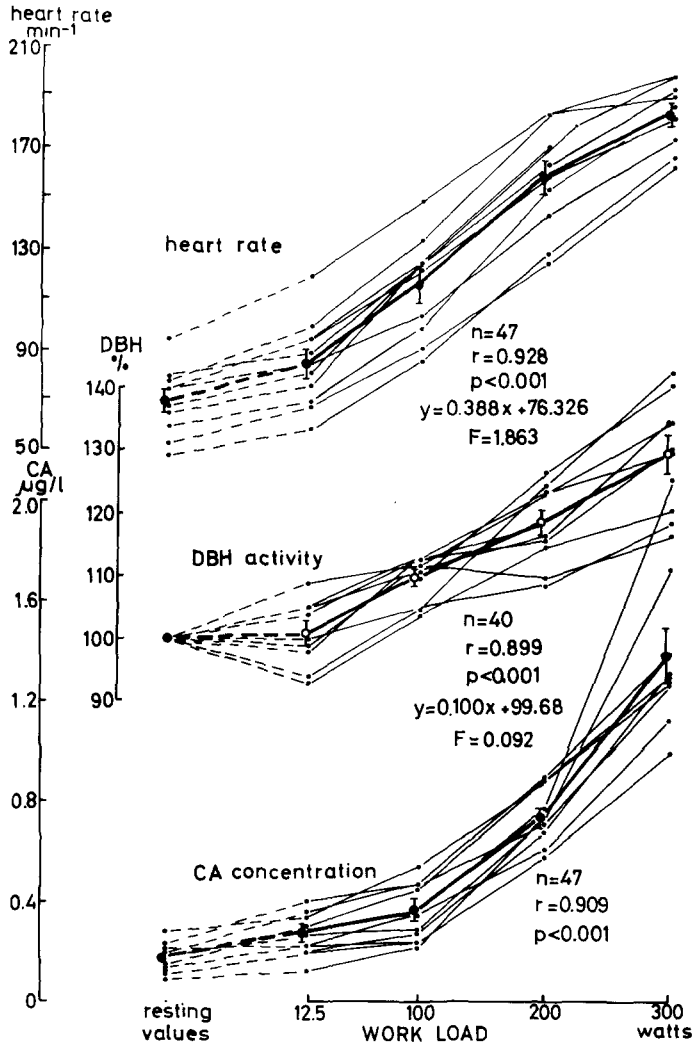

Fig. 2. Correlations between heart rate (min^{-1}), DBH activity (% of resting values), and CA concentrations (µg/l) in plasma and work load

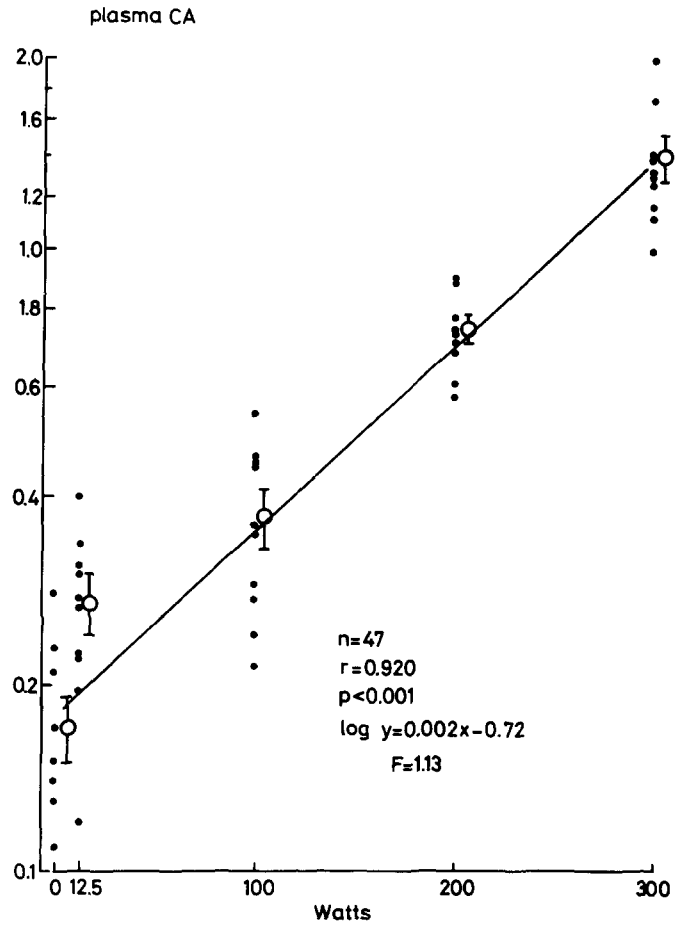

Fig. 3. Correlation between log CA concentration in plasma (ordinate) and work load (abscissa)

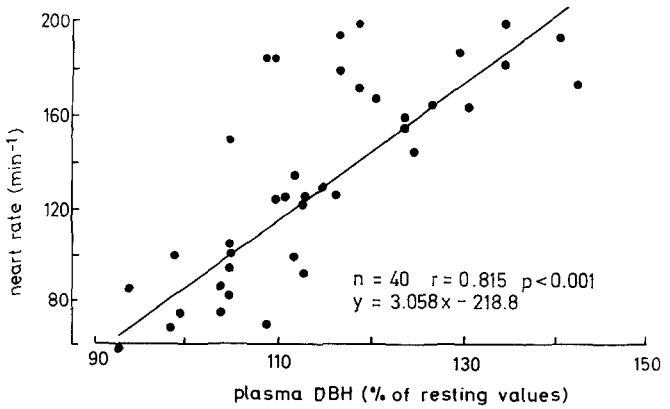

Fig. 4. Correlation between heart rate (ordinate: min $^{-1}$) and DBH activity in plasma (abscissa: % of resting values) during work load (12.5 - 300 W)

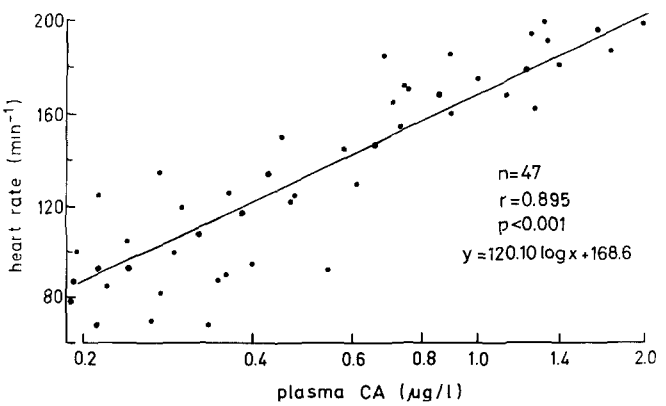

Fig. 5. Correlation between heart rate (ordinate: min^{-1}) and log CA concentration (abscissa: µg/l) during work load (12.5 - 300 W)

cal method, which measures active enzyme as well as enzyme possibly inactivated during diffusion) was greater than the increase in DBH *activity* measured by conversion of tyramine to octopamine.

In addition, enlargement of the circulatory volume should be anticipated because of the change from the supine position during the rest period to the erect position (work load of 12.5 watts, 5 min), i.e. by orthostatic regulation (Schmidt and Schmidt, 1969; Friedel *et al.*, 1973). This may explain why DBH activity sometimes fell after a work load of 12.5 watts for 5 min. Similar results were presented by Wooten and Cardon (1973) immediately after tilt, and have also been obtained by Planz *et al.* (1973; unpublished observations).

2. After work loads between 100 and 300 watts the heart rate and plasma DBH activity increased almost linearly, i.e. in a direct correlation with the corresponding work loads (c.f. Fig. 2). The increases in heart rate and DBH activity were also directly related (Fig. 4).

On the other hand, the slope of the curve for plasma CA concentration became steeper with increasing work load (c.f. Fig. 2). Similar results have been reported by Carlsson *et al.* (1968), Häggendal *et al.* (1970) and Häggendal (1971). From Fig. 3, however, it is evident that there was a linear correlation between the logarithm of CA concentration in plasma and work load. Since the correlations heart rate/work load, DBH/work load and log CA/work load were also linear, it can be concluded, too, that the parameters heart rate/DBH, heart rate/log CA and log CA/DBH were both significantly (c.f. Fig. 4, 5, 6) and linearly correlated.

The exponential rise in CA concentration during increasing work load might be explained by an increasing net "overflow" of CA, i.e. the percentage of noradrenaline released from nerve terminals (which had escaped the various inactivation mechanisms within the synaptic cleft to reach the intravasal space) would increase disproportionally more than anticipated from the corresponding increments in work load. Similar results have been obtained under in vitro conditions, when sympathetic nerves of isolated organs were stimulated

Table 1. Increase of total catecholamines and noradrenaline and adrenaline in human plasma at maximum work load (300 W); p values represent significance of difference from the resting values

	Adrenaline	Noradrenaline	Total Catecholamines
	Method: Passon and Peuler, 1973		Method: Renzini et al., 1970
	ng/l		ng/l
Rest	90 ± 19	176 ± 9	164 ± 16
300 W	137 ± 17[a]	992 ± 99[b]	1448 ± 136[c]
	n = 5	n = 5	n = 8
	[a] $p > 0.05$	[b] $p < 0.01$	[c] $p < 0.001$

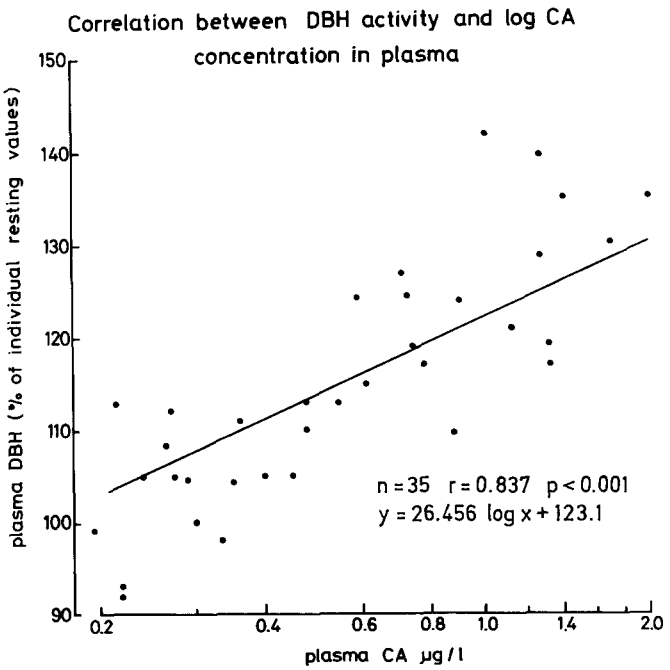

Fig. 6. Correlation between plasma DBH (ordinate: % of resting values) and log CA concentration (abscissa: µg/l) during work load (12.5 - 300 W)

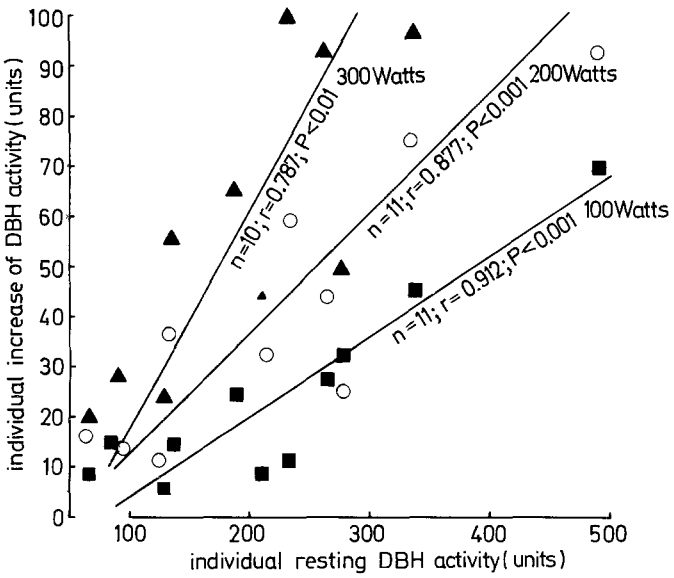

Fig. 7. Proportional increase of DBH activity in plasma (ordinate: units = nmoles octopamine formed/20 min/ml plasma) at three work load steps (■ = 100 W, ○ = 200 W, ▲ = 300 W) related to the corresponding resting level of DBH

electrically at increasing frequencies (Brown and Gillespie, 1957; Huges and Roth, 1971). This phenomenon can readily be accounted for by the assumption that release of noradrenaline is directly proportional to sympathetic nerve activity, and, inactivating mechanisms, although highly efficient at low rates of stimulation, will be overcome at higher frequencies (for review, see Holtz and Palm, 1966); see also 'Discussion', point 4.

3. When DBH activity was measured five minutes after termination of exercise, the enzyme level was as high as immediately after a work load of 300 watts, whereas CA concentrations had already dropped to 30% of their peak values. Again, this characterizes the slow release and/or elimination processes of the enzyme. The simultaneous drop in CA concentration and heart rate points to an immediate decrease of sympathetic activity. The levels of plasma DBH reached half maximum values 22 min after termination of the work load. The elimination of enhanced DBH activity from the plasma, however, did not follow a one compartment-open model, since no log-linear elimination phase could be observed in these experiments. An almost identical half time of 20.6 min was measured for normalization of plasma enzyme activity in 22 volunteers who underwent exercise of diverse severity and who therefore had different peak values of DBH activity, ranging from 108 to 135% of normal. In them log-linear elimination occurred. Nothing is known of the mechanisms of elimination of the enzyme from the plasma, the rate of which, may or may not depend on the height of the stress-induced increase.

From this it may be concluded that a rapid decrease of sympathetic activity in vivo is more precisely reflected by the corresponding fall of plasma CA concentration, whereas an acute increase in sympathetic tone is better mirrored by the enhancement both of DBH activity and CA concentration.

4. Although it has been shown in animal experiments that DBH in plasma is mainly derived from sympathetic nerves and not from the adrenals (Weinshilboum and Axelrod, 1971b; Axelrod, 1972), some contribution of the adrenal medulla to the enhancement of DBH activity and CA concentrations in plasma during physical exercise in man cannot be entirely excluded. Greater CA release from the adrenal medulla by increased physical exercise could explain the disproportionate increase in plasma CA concentration and DBH activity (Fig. 2). For this reason, noradrenaline and adrenaline were determined in plasma specimens after the maximum work load of 300 watts. Because of the difficulty of identifications by the fluorimetric method of adrenaline in the presence of noradrenaline, the radiometric assay of Passon and Peuler (1973) was also used. Almost identical values were found by both methods for total CA concentration in plasma during rest and after a work load of 300 watts (see Table). In addition, there were higher noradrenaline concentrations, depending on the amount of exercise performed (Gray and Beetham, 1957; Kitchen *et al.*, 1971; Banister and Griffiths, 1972). With regard to the increase of plasma adrenaline, the present data are in accordance with those reported by Häggendal *et al.* (1970), Chin and Evonuk (1971) and Chin *et al.* (1973). These authors stated that there was little or no increase in circulating plasma adrenaline with increasing work load (see also Vendsalu, 1960). Banister and Griffiths (1972), however, found increases in plasma adrenaline concentration of more than 300%. From our data, it may be concluded that under the experimental conditions defined above (see "Methods") adreno-medullary activation occurs only to a minor degree. Thus, the DBH activity and CA concentration depicted in the figure appear to reflect exclusively activation of sympathetic nerves. It is known, however, that under certain conditions there is enhanced secretion either of noradrenaline or adrenaline from the adrenal medulla (for review, see Holtz and Palm, 1966).

If, during increasing work load, the effects of noradrenaline (released from the sympathetic nerves) were amplified by adreno-medullary noradrenaline (and not by adrenaline) it could be postulated that adreno-medullary secretion of noradrenaline would not be accompanied by secretion of DBH. This mechanism could also account for the equation: DBH activity = b x log CA + a (Fig. 6). Experimental results obtained recently in man during cold stress have indicated isolated secretion of noradrenaline from the adrenal medulla, which was not accompanied by raised adrenaline concentration and DBH activity in plasma (Appel *et al.*, 1974).

5. Because of the wide range of interindividual variation of DBH activity, changes have been expressed in % of the individual resting values (c.f. 'Methods'). This procedure seems to be correct, since it was shown previously that increases in DBH activity were directly correlated with the appropriate resting value (Planz and Palm, 1973). This was confirmed in the present experiments, in which there were virtually linear correlations between absolute increases of DBH activity (units) and the corresponding absolute resting values (Fig. 7) obtained for each work load (100, 200, 300 watts).

The present results do not agree with those of Freedman *et al.* (1973). The latter authors stated that enhancement of enzyme *concentration* after a cold pressor test did not depend on the magnitude of the resting values. In their experiments, however, DBH *concentrations* in human plasma specimens were assayed by an immunological method, which measures both active and probably inactive protein molecules derived from the released enzyme. Despite this apparent discrepancy, Ebstein *et al.* (1973) have stated that a linear relationship was found when increases in DBH *concentration* in plasma, measured immunologically, were plotted against the corresponding increases in enzyme *activity*, measured by the velocity of conversion of tyramine to octopamine.

From the present experiments, it is apparent that acute increases of sympathetic activity in man can be determined precisely by measurement of the rise of DBH activity in plasma, since a significant

correlation was found between changes in DBH activity and the logarithm of CA concentration. The results also support strongly proportional release of DBH and CA from sympathetic nerve terminals in man due to the process of exocytosis, as already suggested by Johnson *et al.* (1971).

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Note Added in Proof

In contrast to graded physical exercise, infusion of tyramine (3 mg/min) in man leads to a significant increase of catecholamines in plasma, without significant changes of dopamine-β-hydroxylase-activity, implying a nonexocytotic mechanism of release. Oral administration of β-blocking drugs (penbutolol, practolol) did not influence the release of catecholamines by tyramine. However, physical exercise (150 watts) causes a significantly greater elevation of plasma catecholamines during pharmacological beta-blockade than without blockade whereas only a tendency for a greater enhancement of dopamine-β-hydroxylase activity was observed under beta-blockade (Grobecker *et al.*, 1974).