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STIMULATION OF DOPAMINE-β-HYDROXYLASE IN RAT ADRENALS BY REPEATED EXPOSURES TO CARBON DISULPHIDE*

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Abstract—The conversion of dopamine to noradrenaline, measured shortly after exposure to carbon disulphide, is reduced in the adrenals of rats. However, alongside this effect, carbon disulphide produces a gradual increase in the adrenal content of dopamine-β-hydroxylase indicated by the increase of *in vitro* estimated enzyme activity and by the increased *in vivo* conversion of dopamine to noradrenaline observed 24 hr after the ninth exposure. Thus, after repeated exposures, the reduced rate of noradrenaline synthesis detectable immediately after the exposure alternates with the increased rate of synthesis.

Carbon disulphide (CS_2) is a widely used solvent in industry. Epidemiological studies on the chronic toxicity of CS_2, mainly in viscose rayon workers, indicated increased incidence of arterial hypertension [1], coronary heart death [2, 3] and behavioural deviations [4] including higher suicide rates [5]. As catecholamine involvement may be a common factor in such disorders [6–9], catecholamine metabolism seemed a natural target in the investigation of CS_2 toxicity. Early studies indicated that CS_2 inhibits the enzyme dopamine-β-hydrolyase (EC 1.14.17.1; 3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase, β-hydroxylating; DβH) [10] and increases the sensitivity of rats to the stereotypic effect of dopaminergic agonists [11]. The mechanism of inhibition is the chelation of copper, the essential DβH cofactor, by the dithiocarbamate-type metabolites of CS_2 [12]. Inhibition of DβH suggests, however, depressed noradrenaline (NA) synthesis which could hardly explain the development of disorders like arterial hypertension or coronary heart death.

In the present study the effect of repeated CS_2 exposures on adrenal catecholamine metabolism was investigated in rats. It has been found that alongside the depression of catecholamine synthesis, detectable soon after every single exposure to CS_2, there was a gradual increase of DβH content in the adrenals which led after repeated exposure to the alternation of low and high rate of NA synthesis.

MATERIALS AND METHODS

Chemicals. CS_2, analytical reagent, was purchased from BDH Chemicals Ltd. (Poole, Dorset, U.K.). [7-^{14}C]Dopamine (DA), sp. act. 57 mCi/mmole, was purchased from Amersham International Plc (Amersham, Bucks., U.K.). Other chemicals were of the purity grade of reagents generally used in the laboratory.

Experimental procedures. Porton–Wistar male rats, when not in exposure chambers, were caged in groups of six with food and water *ad libitum* and kept at laboratory conditions of light and temperature throughout the time of the experiments. Rats were selected with different initial body weights (120–200 g) to ensure that at the time of sacrifice they were all approximately 200 g despite different exposure times. Rats were exposed daily to a concentration of 2 mg CS_2/l. air for 4 hr one or nine times (on days 1–5 and 7–11) in dynamic inhalation chambers where the concentration of CS_2 was continuously monitored by an infra-red spectrometer [13]. Control rats, paired with experimental ones, were exposed to the same conditions (including air flow) without CS_2 in the atmosphere. At different times after one or nine exposures, controls and CS_2 rats were anaesthetized by an i.p. injection of 60 mg pentobarbitone sodium/kg and were given a pulse injection of [^{14}C]DA (25 μCi/kg, 0.44 μmole/kg) into the tail vein in not more than 0.2 ml 1% saline adjusted to pH 7.4 with concentrated HCl. Rats were killed by decapitation 15 min later and both adrenals were quickly removed, weighed, frozen in liquid nitrogen, and stored at −30° until assay. An additional group of rats was killed after five exposures to CS_2 for DβH determination in the adrenals.

Catecholamine and metabolite assay. Adrenals were homogenized in 1 ml of 0.1 M ice-cold phosphate buffer, pH 7.4, containing 0.1% Triton X-100 by a Ystral laboratory disperser and shaken in an ice bath for 30 min. Tissue extracts were prepared from 0.5 ml of the homogenate according to the method of Atack and Magnusson [14]. The extract was centrifuged at 40,000 *g* for 1 hr at 4° and the supernatant was filtered and analysed.

(A) For the determination of catecholamines, 20–30 μl filtrate was injected into a HPLC system connected to an electrochemical detector [15]. DA, NA, adrenaline (A), 3,4-dihydrophenylglycol (DHPG)

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and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations were calculated by comparing peak heights with those of a solution containing standard amounts.

(B) Filtrate (200 μl) was then injected in the HPLC system, the eluates were collected in 1 ml fractions, and radioactivity was measured in each vial by a liquid scintillation counter. Three peaks of radioactivity were obtained, corresponding respectively to the retention times of NA + A, DA and DOPAC.

DβH assay. 0.3 ml of the original homogenate was mixed with 0.9 ml of 0.1 M phosphate buffer–0.1% Triton X-100, pH 7.4, and centrifuged at 12,000 *g* for 15 min at 4°. DβH activity was measured in the supernatant from the conversion of tyramine to octopamine according to the method of Kato *et al.* [16]. In this method, copper and ethyl-maleimide are added to the incubation mixture and therefore the inhibitory effect of endogenous and exogenous (like the dithiocarbamate-type metabolites of CS_2) copper chelators is eliminated.

RESULTS

No clinical disorders were detectable in the rats up to nine exposures to CS_2, and neither body weight nor the weight of the adrenals was affected by exposure. Carbon disulphide increased the DA and DOPAC contents in the adrenals while the NA, A and DHPG contents were found to be normal or only slightly reduced (Fig. 1). These changes of catecholamine contents did not indicate any cummulative effect as they were approximately at the same level after a single as after nine exposures. In rats exposed nine times, the adrenal content of DA and DOPAC remained above control levels 24 hr after the end of the last exposure but they returned to normal levels within 96 hr.

The adrenal contents of catecholamines and the ratios of NA + A specific activity to DA specific activity immediately after one or nine exposures were in agreement with the inhibition of DβH (Table 1). At least after the first exposure the conversion of DA to DOPAC seemed to be slightly increased suggesting a shift between the two metabolic pathways: hydroxylation and deamination. No reduction in the rate of DA conversion to NA was observed at 24 hr after the ninth exposure. At that time, in spite of the lower specific activity of the larger than normal DA pool, the specific activity of NA + A was the same as in control rats. The increase in the ratio of specific activities of NA + A to DA was indicative of a significantly increased rate of DA-β-hydroxylation *in vivo*. This finding was in agreement with the increased DβH activity measured *in vitro* in the adrenals of rats at the same time, that is 24 hr after the end of the last exposure (Fig. 2). The *in vitro* activity of DβH in the adrenals of rats exposed to CS_2 was not different from that in controls soon after the first exposure but gradually increased above control values with the number of daily exposures. After the last exposure, the *in vitro* activity of DβH started to decline and it was again at control levels within 96 hr.

DISCUSSION

The rate of DA conversion to NA was reduced in the adrenals of rats exposed to CS_2 in agreement with the reported inhibitory effect of CS_2 on DβH.

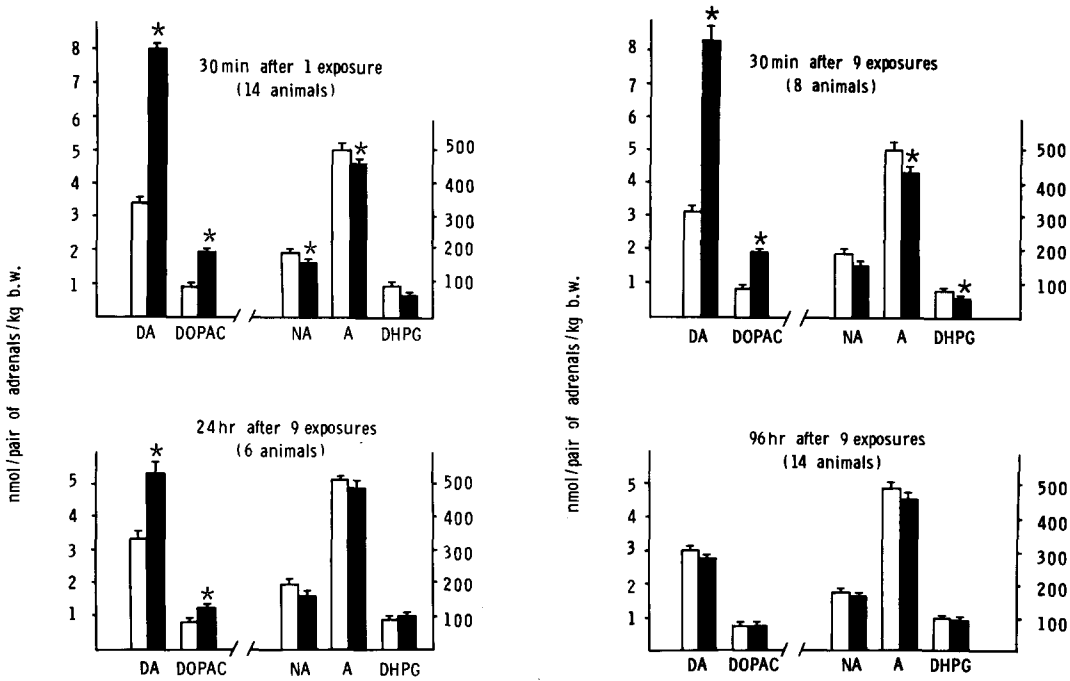

Fig. 1. Catecholamine contents in the adrenals of CS_2-treated rats (solid bars) and controls (unfilled bars) (mean ± S.E.M.). Asterisks mark the significant difference from controls at $P < 0.05$ level (Student's *t*-test).

Table 1. Effect of CS_2 exposure on the specific activities of adrenal catecholamines after the injection of [^{14}C]dopamine

No. of exps.	Time after exps.	No. of rats	Specific activities (% of paired controls (mean ± S.E.M.)) DA	NA + A	DOPAC	Ratios NA + A / DA	DOPAC / DA
1	30 min	8	78 ± 9*	40 ± 5*	87 ± 8	53 ± 5*	114 ± 3*
9	30 min	8	64 ± 2*	44 ± 2*	71 ± 3	70 ± 5*	113 ± 9
9	24 hr	6	75 ± 9*	102 ± 15	87 ± 21	135 ± 6*	116 ± 25
9	96 hr	6	114 ± 12	122 ± 12	120 ± 22	110 ± 10	105 ± 13

* Significant difference at $P < 0.05$ (Student's t-test for correlated groups).

While a significant increase of DA and DOPAC concentrations was detectable after every CS_2 exposure, the contents of β-hydroxylated amines remained unchanged or only slightly reduced throughout the entire experiment. This was an expected finding because of the large pool and slow turnover of NA and A in the adrenals [17] and the lack of cumulative effect on the conversion of DA to NA. Moreover, a gradual elevation of DβH activity measured *in vitro* was detected with the increasing number of exposures. The discrepancy observed soon after exposure between the reduced *in vivo* conversion of DA to NA and the elevated *in vitro* DβH activity after repeated exposures is in agreement with the reactivation of the enzyme during the *in vitro* assay. DβH activity, measured *in vitro*, most probably reflects the total DβH content of the adrenals rather than the actual activity *in vivo*. This view was supported by the finding that 24 hr after the end of the last exposure, when DβH activity *in vitro* was still above control values, the conversion of DA to NA was not reduced but similarly increased, because at that time CS_2 and its active metabolites had been cleared from the body.

In summary, the present results suggest that the depression of NA synthesis, which is an early effect of CS_2 exposure, results after repeated exposures in an increase in the adrenal content of DβH which may ultimately explain the increased NA synthesis 24 hr after the last exposure. However, as at that time, in spite of the increased rate of DA conversion to NA, the DA content of adrenals remained high, it seems very likely that other enzymes involved in the synthesis of catecholamines were also affected. Actually, Heubusch and DiStefano [18] have shown increased *in vitro* activity of tyrosine-hydroxylase in the brains of rats repeatedly exposed to CS_2.

In experimental animals, DβH and tyrosine-hydroxylase are induced by those procedures which increase the level of activity in the sympathoadrenal system, as, for instance: administration of insuline [19], immobilization stress [20], psychological stress [21], and repeated exposures to cold [22]. All these procedures, by increasing catecholamine release from the adrenals, cause a relevant depletion of NA and A stores which has been proposed to play an important role in the initiation of enzyme induction [23]. However, present experiments show that DβH can be induced and catecholamine synthesis increased by repeated exposures to CS_2 in the absence of catecholamine depletion.

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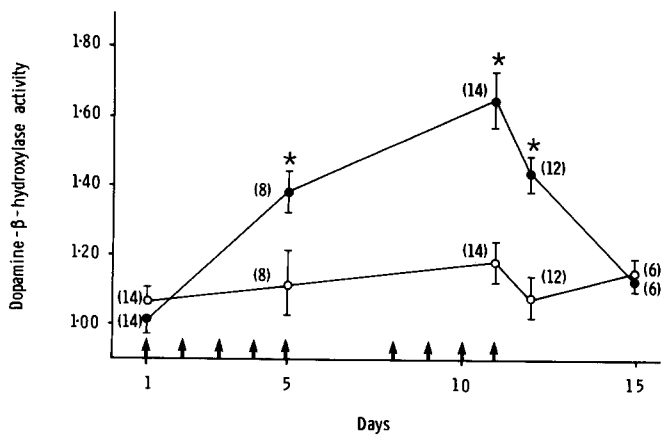

Fig. 2. Adrenal DβH activity (μmole octopamine formed/45 min per pair of adrenals per kg body wt) of CS_2-treated rats (●) and controls (○) (mean ± S.E.M.). Numbers of animals are shown in parentheses. Each arrow represents one 4 hr exposure to 2 mg CS_2/l. air. Asterisks mark the significant differences from controls at $P < 0.05$ level (Student's t-test).

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