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The effects of carbon disulphide exposure on brain catecholamines in rats

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Summary

1. Rats exposed to 2·0 mg/l. carbon disulphide (CS_2) in the inspired air for 2 days, 4 h a day, showed a 13% decrease in their brain noradrenaline concentration and a 16% increase in their brain dopamine concentration.

2. After exposure for 5 or 10 days there was a further decrease in the concentration of noradrenaline in the brain, but brain dopamine returned to the control level.

3. In animals treated intraperitoneally with 2·0 mg/kg reserpine and exposed 2 and 3 days later to 2·0 mg/l. CS_2 for 4 h per day, the brain dopamine concentration showed a 77% increase compared with the unexposed reserpinized animals, but the noradrenaline concentration remained unchanged.

4. The dopamine concentrations in the adrenals after 10 days' exposure to 2·0 mg/l. CS_2 were 67% to 100% higher than in the control animals. In reserpinized rats, 2 days' exposure to CS_2 nearly trebled the dopamine content of adrenals.

5. Exposure to CS_2 had no effect on the tyrosine concentration in the brain, and there was no change in the brain monoamine oxidase (MAO) activity. Tyrosine in the brain showed a 30 to 96% increase in concentration and MAO activity, using kynuramine as substrate, showed an approximately 5% increase 0·5 to 2 h after the subcutaneous administration of 500 mg/kg sodium diethyldithiocarbamate.

6. CS_2 at 10^{-2}M or lower concentrations had no inhibitory effect on the brain MAO activity *in vitro*. Diethyldithiocarbamate inhibited MAO at 10^{-2}M, but not at 10^{-3}M or lower concentrations.

Introduction

Carbon disulphide (CS_2) exposure can lead to widely different pathological conditions such as mental aberrations, hypertension, extrapyramidal symptoms and atherosclerosis (Quarelli, 1937; Gordy & Trumper, 1938; McDonald, 1938; Lewey, 1941a; Paluch, 1948; Vigliani & Pernis, 1955; Weist, 1957; Magos, 1961; Szobor, 1962). Recently, Tiller, Schilling & Morris (1968) have given statistical evidence on the increased frequency of death from coronary heart disease among viscose workers exposed to CS_2 and other toxic agents in this country.

As catecholamines or drugs acting on catecholamine metabolism can influence brain functions and behaviour (Hornykiewicz, 1966; Brodie, Spector & Shore, 1959; Dewhurst, 1968), vasoregulation (Furchgott, 1955), thrombus formation (Rowsell,

Hegardt, Downie, Mustard & Murphy, 1966), lipid metabolism (Wirsen, 1965), the development of cardiovascular lesions (Méhes, Papp & Rajkovits, 1967 ; David, Hecht & Uerlings, 1968 ; Nityanand, 1967 ; Whittington-Coleman, Carrier & Clower, 1968), the effect of CS_2 on catecholamine metabolism might be a common denominator for the development of carbon disulphide intoxication. This hypothesis that CS_2 acts on the metabolism of catecholamines gained support by reports that disulfiram (Goldstein, Anagnoste, Lauber & McKereghan, 1964 ; Musacchio, Kopin & Snyder, 1964) and diethyldithiocarbamate (DDC) (Carlsson, Lindquist, Fuxe & Hokfelt, 1966 ; Edington, 1968) inhibit dopamine-β-hydroxylase, and disulfiram is metabolized through DDC to CS_2 (Johnston & Prickett, 1952 ; Fischer & Brantner, 1967). However, the effect of DDC is not restricted to dopamine-β-hydroxylase. There are reports of its action on tyrosine hydroxylase (Taylor, Stubbs & Ellenbogen, 1969) and monoamine oxidase (MAO) (Edington, 1968). The inhibitory effect of CS_2 on MAO was also reported (Magistretti & Peirone, 1961).

In this study the concentrations of dopamine (DA) and noradrenaline (NA) in the brain were estimated in rats after exposure to 2·0 mg/l. CS_2 in the inspired air. The effects of CS_2 on MAO and the brain tyrosine concentration were also investigated and compared with the effects of DDC.

Methods

Animal experimentation

Male rats of Porton-Wistar strain, 190–220 g in weight, were used.

All the animals used in these experiments were exposed to 2·0 mg/l. CS_2 in a vertical constant flow exposure chamber fed by compressed air at a rate of 5 l./min. CS_2 was injected into the input air by slow injection apparatus (C. F. Palmer Ltd., London) through an atomizer. Carbon disulphide concentration in the chamber was recorded on a Servogor potentiometric recorder (Goertz Electro Ges.m.b., Wien) attached to an infrared spectrometer (Perkin-Elmer Ltd., Beaconsfield) by sampling air through a 10 cm gas cell at a rate of 500 ml/min. The calibration curve for the infrared spectrometer was made by plotting the CS_2 estimated by the method of Viles (1940) against absorbance at a wavelength of 6·5 μm. The variations in concentrations were not more than 10%. Each exposure lasted 4 h and was repeated on successive days, excluding Saturday and Sunday, and the animals were killed within half an hour after the last exposure. Control rats were exposed in a similar chamber without CS_2.

Rats which had been given 2·0 mg/kg reserpine (Sigma Chem. Co., St. Louis, Missouri) intraperitoneally were exposed to CS_2 48 and 72 h later and killed after the second exposure.

Rats were given 500 mg/kg sodium diethyldithiocarbamate (Hopkin & Williams, Chadwell Heath, Essex) subcutaneously and were killed 0·5, 1 and 2 h after the injection. Two Dutch rabbits of 2 kg body weight were given 750 mg/kg DDC in isotonic concentration intravenously. They were killed 2 h after the injection. Paired control rats or rabbits were given saline.

Chemical methods

Catecholamines were estimated by the method of Chang (1964) with the following modifications. (*a*) The supernatant *n*-butanol layer was shaken with 2 : 2 : 4-tri-

methylpentane instead of heptane and the catecholamines were extracted with 0·01 N HCl instead of water; (*b*) the pH of the aqueous phase added to alumina was very carefully adjusted to pH 7·5 with 1 ml 2 M sodium acetate and 5 N NaOH added from a micropipette and mixed with a magnetic stirrer; (*c*) 0·6 ml of 0·1 M EDTA was added to the 3 ml of 0·1 M acetic acid eluate and the pH was adjusted very carefully to 6·5 with 5 N NaOH.

After oxidation, the NA samples were heated for 2 min, cooled and the fluorescence read in an Aminco-Bowman Spectrofluorometer. DA samples were heated for a further 5 min, cooled and the fluorescence read 18–20 h later.

The tyrosine contents of brain homogenates were estimated by the method of Waalkes & Udenfriend (1957). After decapitation the brains were quickly removed, dropped into 10 ml ice cold water and homogenized with a Turrex homogenizer and the volume was made up with water to give a 10% homogenate. 2 ml of the homogenate was immediately mixed with 2 ml water and 1 ml 30% trichloroacetic acid. It was found that the time interval between killing the animals and the acidification of the homogenate is critical. Even brains frozen after removal and kept in deep freeze overnight gave high readings. MAO activity was estimated in 1:50 brain homogenates (w/v) from the appearance of 4-hydroxyquinoline (4HOQ) which arises from the spontaneous (cyclization) of the intermediate aldehyde formed by the oxidative deamination of kynuramine by the method of Krajl (1965). 1 ml homogenate was mixed with 0·5 ml of 0·5 M phosphate buffer, pH 7·4 and 1·0 ml distilled water. After 15 min incubation at 37° C 100 μg kynuramine dihydrobromide was added in 0·5 ml water. When the *in vitro* effect of DDC, CS_2 or pargyline hydrochloride was studied on brain MAO activity 1 ml of dilutions of these compounds in water was substituted for 1 ml of distilled water to give final concentrations ranging from 10^{-2} to 10^{-6}M. Some of the experiments were repeated using 5-hydroxytryptamine and tyramine as substrate. The method then used was essentially that of Sjoerdsma, Smith, Stevenson & Udenfriend (1955). 5-Hydroxytryptamine was measured by the colorimetric method of Udenfriend, Weissbach & Clark (1955) after precipitation with trichloroacetic acid instead of the *n*-butanol extraction. Tyramine was measured by the method of Udenfriend & Cooper (1952).

Results

Table 1 shows that CS_2 exposure affected both NA and DA concentrations in the brain. After two exposures brain NA decreased by 13% and DA increased by 16%. After exposure for 5 or 10 days DA concentration returned to the control value, but

TABLE 1. *Effects of exposing rats to 2·0 mg/l. CS_2 in inspired air on brain noradrenaline and dopamine concentrations with or without reserpine treatment*

Exposure	Reserpine pretreatment	No.	Noradrenaline	Dopamine
2×4 h	–	12	87·58 (±3·76)%*	116·08 (±2·78)%*
5×4 h	–	12	70·08 (±2·27)%*	101·08 (±2·90)%
10×4 h	–	12	67·67 (±2·33)%*	102·25 (±5·16)%
2×4 h	+	5	99·10 (±13·32)%	176·60 (±25·21)%†

In one group of rats 2·0 mg/kg reserpine was given intraperitoneally and 48 and 72 h later the animals were exposed to CS_2. Catecholamine concentrations are expressed as the percentage of the paired controls. The figures in parentheses are the standard errors of the means. The absolute value for the noradrenaline concentrations in control brains was 0·374 (±0·011) μg/g and for dopamine 0·678 (±0·019) μg/g. In reserpine treated animals the control values were 0·057 (±0·0039) μg noradrenaline and 0·149 (±0·0074) μg/g dopamine.

* Significant difference $P \ll 0{\cdot}001$. † Significant difference $P < 0{\cdot}025$.

NA concentrations showed a further decrease, and after 10 days the brain of the exposed animals contained 32% less NA than the brains of the controls.

After ten exposures the adrenals of four control and four exposed animals were also tested for DA. The adrenal DA concentrations were 67 to 100% higher in the exposed animals than in the paired controls.

Table 1 also shows that when the animals were given reserpine 2·0 mg/kg intraperitoneally and twice exposed to CS_2 48 and 72 h later, the increase in DA concentration was speeded up without any effect on the very low concentration of NA. The DA content of adrenals was estimated in four reserpinized exposed animals and showed a 170% increase compared with the paired controls.

Table 2 shows that exposure to CS_2 had not influenced MAO activity and the brain tyrosine concentrations were also unchanged. In this respect it seems that the effect of DDC differs from the effect of CS_2. Table 3 shows that after the administration of 500 mg/kg DDC, MAO activity using kynuramine as substrate slightly but significantly increased and the brain tyrosine concentrations showed a rapid increase. MAO activity in two rabbits injected intravenously with 750 mg/kg DDC showed a 10% decrease in the brain compared with the controls. Activation of MAO was not observed in the brain of rats killed one hour after the administration of 500 mg/kg DDC when 5-hydroxytryptamine or tyramine was used as substrate. The differences between the MAO activity in the brains of six control rats and six treated rats were 2·0% with 5-hydroxytryptamine and none with tyramine.

Figure 1 shows the *in vitro* effects of CS_2, DDC and pargyline hydrochloride on MAO activity in rat brain homogenates using kynuramine as substrate. It can be seen that pargyline even in a concentration of 10^{-5}M completely inhibited MAO; DDC caused no inhibition at 10^{-3}M but 42% inhibition at 10^{-2}M. CS_2 had no

TABLE 2. *Effect of exposing rats to 2·0 mg/l. CS_2 in inspired air on monoamine oxidase activity and tyrosine concentration in the brain*

Exposure	No.	MAO activity	Tyrosine concentration
2×4 h	6	98·0 (±2·477)%	—
5×4 h	6	97·0 (±2·967)%	—
10×4 h	8	96·87 (±1·540)%	98·25 (±6·261)%

Monoamine oxidase activity and tyrosine concentrations are expressed as the percentage of the paired controls. The figures in parentheses are the standard errors. The value for monoamine oxidase activity calculated from the appearance of 4-hydoxyquinoline (4HOQ) from kynuramine for the control brains was 7·45 μmol 4HOQ/g per h (±0·056 S.E.M.) and for the concentration of tyrosine it was 18·162 (±0·769 S.E.M.) μg/g tissue.

TABLE 3. *Effect of subcutaneous sodium diethyldithiocarbamate on MAO activity and tyrosine concentration in the rat brain*

Time after injection (h)	MAO activity	Tyrosine
0·5	106·65 (±1·350)%† (N=10)	160·40 (±6·400)%† (N=5)
1	104·56 (±1·316)%* (N=9)	196·60 (±4·932)%† (N=5)
2	105·75 (±1·344)%* (N=10)	130·00 (±8·660)%* (N=5)

500 mg/kg DDC was injected subcutaneously. MAO activity and tyrosine concentrations are expressed as the percentage of the paired controls. The figures in parentheses are the standard errors of the means. The absolute value in controls for MAO activity measured from the conversion of kynuramine to 4-hydroxyquinoline was 6·40 (±0·064) μmol 4 HOQ/g per h and for tyrosine concentration it was 17·24 μg/g.
* $P<0·025$. † $P\ll0·0005$.

inhibitory effect even at this very high concentration. Identical curves were obtained with 5-hydroxytryptamine and tyramine as substrates. Rabbit brain behaved like rat brain.

Discussion

The 2·0 mg/l. concentration of CS_2 used in these experiments was high compared with the 60 μg/l. threshold limit recommended in the U.S.A. for occupational exposure. However, the short term exposure of the rats must be compared with the exposure of workers to CS_2 for months and years. The exposure in our study was in the same range as CS_2 exposures used by other investigators who observed serious neurological disorders and vascular damage in animals. Dogs, after 11 weeks' exposure to 1·2 mg/l. CS_2, developed behavioural changes, rigidity, tremor, paralysis and retinal angiospasms (Lewey, 1941b). In rabbits after 16 weeks of exposure to 1·5 to 2·25 mg/l. CS_2, Cohen, Scheel, Kopp, Stockell, Keenan, Mountain & Paulus (1959) found interstitial nephritis, mild fatty degeneration on the liver, meningeal swelling and infiltration, cerebral and cerebellar lesions involving individual nerve cells and also enlarged adrenals. Glomerulosclerosis was observed in rats after exposure for one year to 2·0 mg/l. CS_2 (Isler, 1957). A complete inhibition of brain MAO of rats exposed to 1·0 mg/l. CS_2 for 4 h was reported by Magistretti & Peirone (1961), who suggested that the thiol groups of this enzyme are extremely sensitive to CS_2.

In our work we were not able to confirm the findings of Magistretti & Peirone (1961). Two to ten 4 h exposures of rats to 2·0 mg/l. CS_2 failed to influence

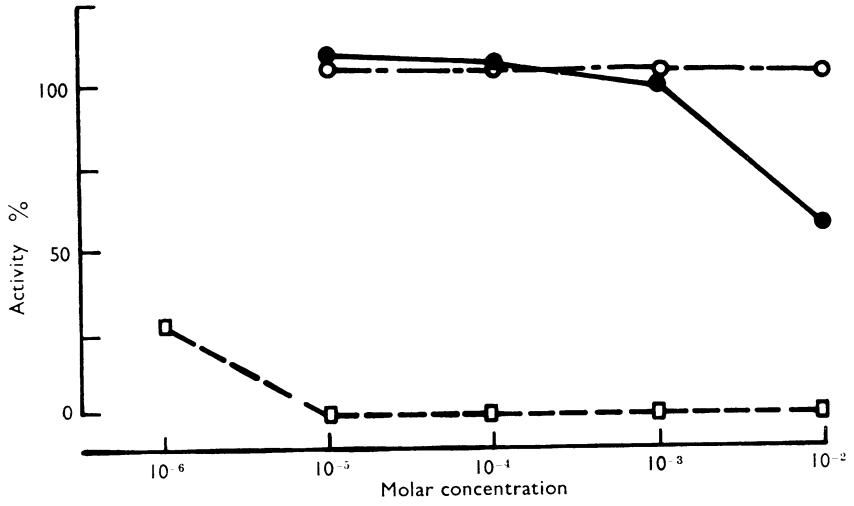

FIG. 1. Effects of carbon disulphide, diethyldithiocarbamate and pargyline on the *in vitro* MAO activity of rat brains. 1 ml various dilutions of CS_2 (○——○), DDC (●——●) or pargyline hydrochloride (□——□) were added to 1 ml 2% homogenate (w/v) and 0·5 ml of 0·5 M phosphate buffer, pH 7·4. The mixture was incubated for 15 min before the addition of 100 μg kynuramine hydrobromide in 0·5 ml water. Final concentrations of the inhibitors in the solution are shown on the abscissa. The absolute value for MAO activity measured from the appearance of 4-hydroxyquinoline (4HOQ) in control experiments was 8·45 (S.E.M. = 0·033) μmol 4 HOQ/g per h. Activities are shown as % of the control values. Every point represents three separate estimations.

brain MAO activity and no inhibition was observed when CS_2 was added to homogenates of rat or rabbit brain up to a concentration of 10^{-2}M. Because of the possible metabolic relation between CS_2 and dithiocarbamates, the effect of DDC on MAO activity was also tested.

DDC added to brain homogenates from rats and rabbits inhibited MAO at 10^{-2}M concentration, but there was no inhibition when the concentration was 10^{-3}M or less. In rabbits 750 mg/kg DDC given intravenously inhibited MAO, but the inhibition measured by us was much less than that observed by Edington (1968). In contrast to the *in vitro* results and the *in vivo* inhibition observed in rabbits, DDC given subcutaneously to rats in a dose of 500 mg/kg slightly but significantly increased the activity of MAO when kynuramine was used as substrate.

The lack of a similar effect after CS_2 *in vivo* and *in vitro* suggests that a metabolite of DDC other than CS_2 was responsible for the *in vivo* effect on MAO. It is more difficult to understand the lack of activation when 5-hydroxytryptamine or tyramine was used as a substrate. It has been known that there are some MAO inhibitors which selectively inhibit the oxidative deamination of various monoamines (Gorkin & Orekhovitch, 1967). The metabolic product of DDC might selectively activate the enzyme or might react with a selective endogenous inhibitor, or interfere with the formation of 4-hydroxyquinoline from kynuramine without affecting the enzyme.

The second effect which was observed in rats after treatment with DDC but not after exposure to CS_2 was the change in the brain tyrosine concentration. Diethyldithiocarbamate, as reported by Goodchild (1969) and confirmed by our study, increased the concentration of tyrosine in the brain. This effect is probably due to the inhibition of tyrosine hydroxylase by the sudden accumulation of NA precursors as DDC either does not inhibit this enzyme (Nagatsu, Levitt & Udenfriend, 1964) or it is much less effective than the iron chelating agents (Taylor *et al.*, 1969). Though the experiments reported here have proved that exposure to CS_2 had the same effect on the NA and DA contents of brain as a single dose of DDC, no change in the brain content of tyrosine was observed after CS_2 exposure. In contrast to the single dose of dithiocarbamate injected into animals, the formation of dithiocarbamates in the CS_2 exposed animals continues for a considerable time.

First CS_2 is taken up only gradually during the exposure and second the half-life of the reaction of CS_2 with amino groups proceeds in the body with a half-life of 6·5 h (Souček & Madlo, 1956).

The experiments reported here gave evidence that after two days of exposure to 2·0 mg/l. CS_2 brain NA decreased and DA increased. Similar changes were observed after treatment with a single dose of DDC (Carlsson *et al.*, 1966 ; Edington, 1968), suggesting that CS_2, like DDC, inhibits the enzyme which converts DA into NA. If exposure followed reserpine treatment the filling up of DA stores in the brain or in the adrenals was much faster than in the control animals, without any effect on the NA concentration. It has been reported that reserpine decreases the binding capacity of the storage particles so that NA is exposed to MAO (Clarkson, 1966 ; Kopin & Gordon, 1962) and actually increases the turnover rate of NA (Neff & Costa, 1968). Increased turnover or the impairment of the capacity of particles to bind NA might explain why the NA concentration remains unchanged in exposed reserpinized animals in spite of the significant increase in the dopamine concentration.

After 5 or 10 days' exposure there was a further decrease in the brain NA, but brain DA returned to the normal value. In the adrenals, however, DA content was nearly doubled after 10 days' exposure. The return of the DA content in the brain to normal suggests that the inhibition of dopamine-β-hydroxylase might not be the sole factor in the observed effects on catecholamine metabolism. Both the activation of MAO and the inhibition of the biosynthetic pathway of DA can counteract the effect of dopamine-β-hydroxylase inhibition on the concentration of DA. As MAO was not activated and the brain tyrosine concentration remained unchanged, the most likely target for CS_2 seems to be the enzyme DOPA-decarboxylase. Nevertheless, it must be pointed out that if the change in the enzyme activity *in vivo* is caused by a change in the concentration of substrate or product, *in vitro* measurement might not reveal the true *in vivo* situation. Consequently the lack of difference in the MAO activity of control and exposed animals does not exclude the possibility that the metabolism of NA or DA due to the activity of this enzyme in the two groups did differ from each other.

These results do not allow any conclusions to be drawn about the mechanisms of CS_2 intoxication, but suggest the need for further research on the effect of CS_2 on catecholamine metabolism. This industrial poison is able to produce different but not specific pathological conditions, of which mental disorders, hypertension, atherosclerosis are in the foreground of medical interest. It might be that regulation disorders brought about by CS_2 simulate the disorder which, without exposure, can lead to hypertension.

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