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# SHORT COMMUNICATIONS

## Duration of the effect of disulfiram on incorporation and metabolism of dopamine-$^{14}$C in hypertensive rat

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DISULFIRAM [bis(diethylcarbamoyl)disulfide], in the presence of ethanol,[1] produces in man toxic effects such as severe hypotension and hyperventilation. Recently Goldstein *et al.*[2] have shown that disulfiram markedly inhibits dopamine-$\beta$-hydroxylase *in vitro* and *in vivo*, probably after reduction of disulfiram by ascorbate to form diethyldithiocarbamate. The inhibition of dopamine-$\beta$-hydroxylase with disulfiram was also demonstrated *in vivo* by Musacchio *et al.*[3] The present study describes the degree and duration of disulfiram-induced inhibition of norepinephrine synthesis from dopamine-$^{14}$C in selected organs in the hypertensive rat.

### METHODS

Animals made hypertensive by DOCA, about 5 months old, were injected i.p. with 125 mg disulfiram/kg (suspended in 5% gum acacia) and killed by cervical dislocation at various times after disulfiram administration. Dopamine-1-$^{14}$C (sp. act. 6·0 mc/m-mole) was injected i.m. at 0·31 mg/kg, 1 hr prior to killing. The analytical procedure for the determination of catecholamines will be presented in detail in another communication and will be described here only briefly. The excised organs were immediately frozen on dry ice, weighed, and homogenized in ethanol-HCl. The precipitated proteins were separated from the supernatant by centrifugation. Aliquots of the supernatant fraction were counted in a Packard Tri-Carb liquid scintillation counter to determine the content of radioactivity in tissues. The remaining supernatant was concentrated to dryness at low temperature under high vacuum. The dry samples were dissolved in ethanol and spotted on Whatman no. 1 paper for descending chromatography. n-Butanol equilibrated with 1 N HCl (1 : 1) was used routinely as the solvent system. Carriers of dopamine, norepinephrine, and their corresponding 3-O-methyl derivatives were spotted simultaneously with the unknown sample (for identification purposes). On occasion, separate chromatograms were developed in a phenol : $H_2O$ (88 : 12) system in order to confirm the identification of the catecholamines and their metabolites.

The developed chromatographic paper strips were scanned in a $4\pi$ chromatogram scanner (Vanguard) for visual analysis of results. For quantitative evaluation of the radioactive peaks, paper-strip zones corresponding to dopamine, norepinephrine, and various metabolites were cut out, placed in a scintillation medium, and counted.

### RESULTS AND DISCUSSION

*Radioactivity content in tissues*

The concentration of radioactivity in the heart, spleen, kidney, and adrenals at different time intervals after disulfiram administration is presented in Table 1. The results, compared with control values, showed no apparent difference in tissue concentration of radioactivity for at least a 6-hr period after disulfiram administration (with one exception: spleen at the 2-hr period). After the 24- and 48-hr periods, the radioactivity content in the kidney was significantly less than control values. The reason for the decrease in radioactivity at the later time period is unclear.

*Effect of disulfiram on dopamine metabolism*

The effect of disulfiram on the metabolic pathway of dopamine-$^{14}$C in the spleen is shown in Fig. 1, which represents a typical chromatographic pattern for this organ. The positions on the paper strip of authentic dopamine (DA), norepinephrine (NE), 3-O-methyl dopamine (no. 3) and two other metabolites (1 and 2), not identified at present, are indicated in this figure. The quantitative evaluation

TABLE 1. EFFECT OF DISULFIRAM ON TISSUE CONTENT OF RADIOACTIVITY AFTER ADMINISTRATION OF DOPAMINE 1-$^{14}$C*

| Tissue | Control | Time after disulfiram administration | | | | |
|---|---|---|---|---|---|---|
| | | 1 hr | 2 hr | 6 hr | 24 hr | 48 hr |
| Heart | 8·31 ± 0·45 (7) | 8·62 ± 1·28 (4) | 9·04 ± 1·33 (5) | 8·80 ± 0·94 (5) | 7·88 ± 1·09 (5) | 6·53 ± 0·70 (4) |
| Spleen | 8·43 ± 0·29 (6) | 7·55 ± 1·45 (4) | 5·67† ± 0·52 (5) | 8·48 ± 0·33 (5) | 9·06 ± 1·33 (5) | 10·19 ± 0·52 (4) |
| Kidney | 55·80 ± 7·55 (6) | 67·53 ± 18·59 (4) | 44·37 ± 17·95 (5) | 70·10 ± 12·75 (5) | 24·48† ± 8·37 (5) | 23·63† ± 7·43 (4) |
| Adrenal | 17·14 ± 2·76 (7) | 14·99 ± 2·82 (4) | 16·31 ± 3·85 (5) | 17·44 ± 2·04 (5) | 10·66† ± 1·49 (5) | 10·94 ± 0·84 (3) |

* Mean values (± S.E.) are presented as counts × $10^3$/min/g tissue. The values in parentheses indicate the number of animals tested.

† Values statistically significantly different from control ($P < 0·05$, Student's $t$ test).

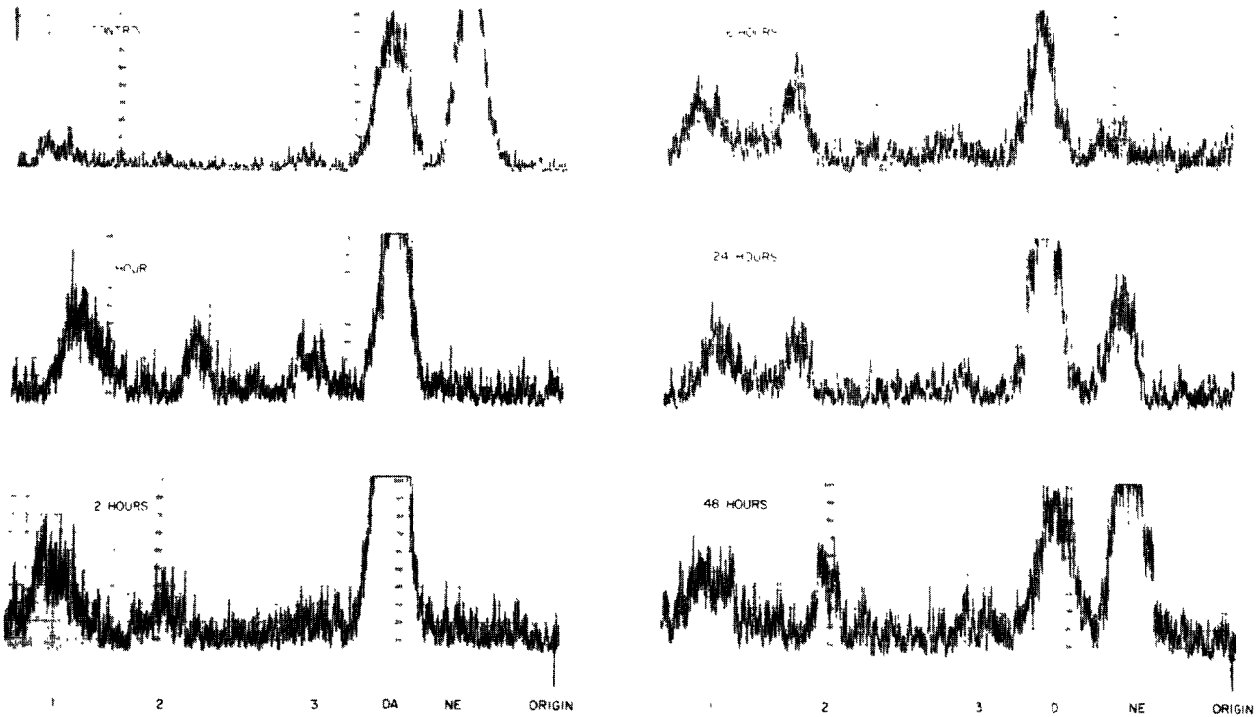


FIG. 1. Inhibition of norepinephrine synthesis with disulfiram in spleen.

TABLE 2. EFFECT OF DISULFIRAM ON CATECHOLAMINE METABOLISM IN RAT SPLEEN*

| | Control | Time after disulfiram administration | | | | |
|---|---|---|---|---|---|---|
| | | 1 hr | 2 hr | 6 hr | 24 hr | 48 hr |
| Norepinephrine (NE) | 49·9 ± 4·35 | 2·1 ± 0·98 | 1·7 ± 0·17 | 7·7 ± 1·81 | 21·8 ± 1·69 | 33·5 ± 5·62 |
| Dopamine (DA) | 33·3 ± 0·92 | 63·6 ± 4·40 | 59·8 ± 3·51 | 45·0 ± 4·05 | 49·3 ± 2·86 | 32·7 ± 5·39 |
| Metab. 3 (3MD) | 3·0 + 0·68 | 4·0 ± 2·33 | 2·3 ± 1·15 | 3·8 ± 1·08 | 1·7 ± 1·03 | 3·8 ± 1·56 |
| Metab. 2 | 1·6 ± 1·04 | 8·5 ± 1·43 | 8·1 ± 0·23 | 16·3 ± 3·90 | 12·8 ± 2·05 | 11·2 ± 1·60 |
| Metab. 1 | 12·1 ± 3·58 | 21·7 ± 2·86 | 28·0 ± 3·66 | 27·2 ± 2·78 | 14·4 ± 2·55 | 18·7 ± 1·94 |
| % Inhibition of NE synthesis† | 0 | 95·8 | 96·5 | 84·6 | 56·3 | 32·8 |

* Results are expressed as per cent of total counts on individual chromatographic strips.
The number of samples analyzed was the same as shown in Table 1.
† $100 - \left(\frac{\%\text{ NE found with disulfiram}}{\%\text{ NE found without disulfiram}} \times 100\right)$.

TABLE 3. EFFECT OF DISULFIRAM ON CATECHOLAMINE METABOLISM IN RAT HEART*

| | Control | Time after disulfiram administration | | | | |
|---|---|---|---|---|---|---|
| | | 1 hr | 2 hr | 6 hr | 24 hr | 48 hr |
| Norepinephrine (NE) | 44·8 ± 3·39 | 4·2 ± 1·82 | 2·0 ± 0·18 | 5·3 ± 1·54 | 15·1 ± 2·55 | 33·7 ± 1·91 |
| Dopamine (DA) | 29·3 ± 3·96 | 53·2 ± 4·83 | 65·3 ± 3·10 | 46·0 ± 3·96 | 53·1 ± 5·56 | 33·3 ± 3·82 |
| Metab. 2 | 3·8 ± 1·34 | 3·1 ± 1·07 | 5·1 ± 0·45 | 14·8 ± 3·94 | 3·4 ± 2·23 | 6·2 ± 2·40 |
| Metab. 1 | 22·0 ± 2·21 | 39·5 ± 5·52 | 27·6 ± 2·79 | 33·4 ± 5·02 | 28·4 ± 5·63 | 26·7 ± 2·04 |
| % Inhibition of NE synthesis* | 0 | 90·6 | 95·4 | 88·2 | 66·3 | 24·6 |

* Conditions as in Table 2.

of the results in Fig. 1 are shown in Table 2. The data clearly show that a single injection of disulfiram (125 mg/kg) strongly inhibits the formation of norepinephrine from its radioactive precursor dopamine at all time intervals tested. The inhibition was at a maximum at approximately 2 hr and was still apparent at the end of the 48-hr period. Disulfiram caused a reduction in norepinephrine and an increase in dopamine tissue levels. In addition, the relative amounts of metabolites 1 and 2 increased appreciably after disulfiram. Little change was observed in the radioactive metabolite 3, corresponding on the paper strip to the position of 3-O-methyl dopamine.

The results obtained in heart (Table 3) show that the degree and duration of inhibition of norepinephrine synthesis with disulfiram was similar to that previously shown in spleen. Higher levels of metabolite 1 were observed in treated animals. The levels of metabolite 2 were quite variable, and metabolite 3 could not be detected in the heart.

Musacchio *et al.*[3] have studied the effect of disulfiram in the heart up to 1 hr after dopamine administration. Goldstein *et al.*[2] reported the inhibition of norepinephrine synthesis with disulfiram in heart and spleen only for a period up to 4 hr. The authors however, injected a total of 400 mg disulfiram/kg 2 hr prior to, and then simultaneously with, the injection of dopamine-$^{14}$C; thus the degree and duration of disulfiram inhibition cannot be properly evaluated.

The data presented in this investigation have shown that a single injection of disulfiram strongly inhibits the formation of norepinephrine from the radioactive precursor dopamine in spleen and heart over a period of at least 48 hr. The inhibition of norepinephrine is accompanied by increasing levels of dopamine, and in spleen also by increasing levels of some (at present) unidentified metabolites.

The action of disulfiram, through inhibition of dopamine-$\beta$-hydroxylase,[2, 3] results in increasing tissue concentration of dopamine which may act as an adrenergic transmitter.[4] If it is assumed that dopamine can act as a less potent transmitter than norepinephrine and that it is released instead of norepinephrine, then it should manifest itself as a reduction in blood pressure in a sensitive test animal such as the DOCA-hypertensive rat. This possibility is now being investigated and will be the subject of a separate report.

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## 1,2,5-Selenadiazoles: A new class of highly cytotoxic compounds

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Although the 1,2,5-selenadiazole ring is known as part of fused-ring systems such as the 2,1,3-benzoselenadiazoles[1] and the [1,2,5] selenadiazolo]3,4-*d*] pyrimidines,[2, 3] the only synthesis of monocyclic 1,2,5-selenadiazoles appears to be that reported in the dissertation of Shew.[4] We have synthesized 4-amino-1,2,5-selenadiazole-3-carboxylic acid (NSC 84531) and several of its derivatives and have found that certain of these selenadiazoles were highly cytotoxic.