*Results and discussion.* It was found that meditation produced a general increase in salivary minerals (Table) especially Na (70%), Mg (42%), Ca (36%) and $P_i$ (46%). However K was increased a lesser amount (23%) and Zn was not significantly altered. 10 min after meditation there was no difference, from control period, in any of the electrolytes. The protein content of the saliva was also increased during meditation. TCA-soluble protein was elevated by 93% and TCA-insoluble protein by 36%, leading to an overall increase of 60% in total protein. 10 min after meditation the total protein content was still slightly elevated (+9%) due mainly to the 23% elevation in the TCA-insoluble fraction. However, the acid-soluble protein was significantly decreased (−12%) at this time. Salivary pH was decreased (0.4 pH unit) during meditation and slightly, but significantly increased (0.1 pH unit) 10 min after the practice. Values obtained for salivary pH and mineral content are in general agreement with published values[6].

The salivary changes during transcendental meditation indicate that extracellular fluid electrolytes may also be altered during this state. Some of the increase in solids is undoubtedly due to water reabsorption and/or the secretion of a more concentrated saliva. However, the large difference in the degree of concentration of solids (from +11% for Zn to +93% for TCA-soluble protein) indicates more than just an overall change in water concentration. Also, differential increase in acid-soluble over acid-insoluble protein, and the fact that the former is decreased 10 min after meditation, while the latter remains elevated, indicates a specific process involving these substances. Decreased salivary pH may reflect a slight acidosis as demonstrated by WALLACE et al.[3].

Studies are continuing on the biochemical correlates of transcendental meditation and the role of mineral nutrition in the mechanisms of consciousness.

*Résumé.* Lors de la pratique de la «méditation transcendantale» on a observé des modifications quantitatives du pH ainsi que des concentrations en électrolytes et protéines de la salive. Après 20 min de méditation on a trouvé des variations de certains minéraux salivères: Na (+70%), Mg (+42%), Ca (+36%) et phosphore minéral (+46%) alors que K et Zn n'ont augmenté que très peu. La concentration en protéines a augmenté et le pH a diminué pendant l'exercice. La composition de la salive est redevenue normale au bout de 10 min après la fin de la méditation.

LARRY W. MCCUAIG

*Department of Biochemistry, Faculty of Medicine, Dalhousie University, Sir Charles Tupper Medical Building, Halifax N.S. (Canada B3H 4H7),*
*18 March 1974.*

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## A Study of the Inhibition by Cysteamine on the Steroid 11 β-Hydroxylation

Recently, it was found that the adrenostatic effect of cysteamine on the corticosterone biosynthesis was caused by an inhibition of the 11β-hydroxylase system in the adrenal cortex[1–3]. With respect to this effect, cysteamine is comparable to metyrapone[4] and similar aromatic substances[5]. This conformity is surprising because cysteamine is aliphatic and has a free sulfhydryl (SH)-group with reducing properties. Therefore, one would assume that the mechanism of inhibition by cysteamine must be different from that by metyrapone.

As a first point of reference for the action mechanism, the inhibition of the steroid 11β-hydroxylase system by cysteamine was quantitatively compared with that caused by metyrapone. Further experiments were based on the fact that the metyrapone inhibition of the 11β-hydroxylase is specific, as the steroid C21 hydroxylase is unaffected[6,7]. Therefore, cysteamine and metyrapone were also compared with respect to their influence on the C21 hydroxylation. In a third series of experiments, cysteamine was compared with chemically related substances as to the effect on the 11β-hydroxylase.

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Table I. Steroid 11β-hydroxylase inhibition by cysteamine and metyrapone

| Inhibitor | Enzyme activity | Concentration of inhibitor (*M*) Control | $0.4 \times 10^{-4}$ | $1.0 \times 10^{-4}$ | $2.0 \times 10^{-4}$ | $4.0 \times 10^{-4}$ |
|---|---|---|---|---|---|---|
| Metyrapone | absolute | 28.61 ± 0.99 | 15.02 ± 0.7[c] | 9.61 ± 0.63[b] | 7.39 ± 0.44[b] | 5.64 ± 0.2 |
| | % | 100 | 53 | 34 | 26 | 20 |
| Cysteamine | absolute | 28.61 ± 0.99 | 20.49 ± 0.62 | 13.80 ± 0.74 | 9.46 ± 0.67 | 6.23 ± 0.45 |
| | % | 100 | 73 | 44 | 33 | 22 |

Enzyme activity, calculated for n*M*/mg protein/h (absolute) and percent of control value. Adrenal homogenates, 4 incubations per dose. Mean ± S.D. *t*-test. All experimental values are significantly different from control values with $P < 0.001$. Significance of difference between metyrapone and cysteamine: [b] $P < 0.01$; [c] $P < 0.001$.

Table II. Inhibition of steroid 11$\beta$-hydroxylase by various sulfhydryl substances

| Substance | Functional groups | Enzyme activity | Control | $4\times10^{-4}$ M | $4\times10^{-3}$ M |
|---|---|---|---|---|---|
| 1. Cysteamine (12) | C–$NH_2$ / C–SH | Absolute | 18.79 ± 1.32 | 4.81 ± 0.68 | 1.29 ± 0.42 |
| | | % | 100 | 26 | 7 |
| 2. 2-Aminoethanol (8) | C–$NH_2$ / C–OH | Absolute | 21.33 ± 2.24 | 21.78 ± 2.02 | 20.47 ± 1.60 |
| | | % | 100 | 102 | 96 |
| 3. Mercaptoethanol (6) | C–OH / C–SH | Absolute | 24.98 ± 3.61 | 21.64 ± 2.5 | 22.68 ± 1.26 |
| | | % | 100 | 87 | 91 |
| 4. Cystamine (8) | C–$NH_2$ / C–S / C–S / C–$NH_2$ | Absolute | 16.97 ± 0.69 | 5.62 ± 0.51 | 2.04 ± 0.16 |
| | | % | 100 | 33 | 12 |
| 5. Cysteine (8) | COOH / C–$NH_2$ / C–SH | Absolute | 18.18 ± 1.22 | 17.27 ± 1.78 | 17.79 ± 0.92 |
| | | % | 100 | 95 | 98 |
| 6. 1,2-Ethandithiol (8) | C–SH / C–SH | Absolute | 22.58 ± 0.97 | 15.78 ± 0.79 | 6.12 ± 0.74 |
| | | % | 100 | 96 | 27 |
| 7. 2,3-Dimercaptopropanol (8) | $CH_2OH$ / C–SH / C–SH | Absolute | 16.97 ± 0.69 | 13.23 ± 2.9 | 7.45 ± 0.47 |
| | | % | 100 | 80 | 44 |

Figures in brackets = number of incubations per dose. All experimental values are significantly different from the values for cysteamine Otherwise see Table I.

*Materials and methods. Enzyme activity.* 1. Homogenates. 4 adrenals from 2 rats (SIV Kisslegg) were used for one homogenate.

2. Steroid 11$\beta$-hydroxylase. The enzyme activity was measured by the change in fluorescence due to increasing concentrations of corticosterone that indicate the rate of hydroxylation of deoxycorticosterone (DOC). The determinations were carried out according to ROSENTHAL and NARASIMHULU[7] and NETTER et al.[8]. The adrenal homogenates were prepared in isotonic sucrose solution (0.25 *M* sucrose; 0.02 *M Tris*; 0.005 *M* EDTA). An aliquot of the homogenate containing 2 mg protein was incubated with shaking for 60 min at 37 °C in 1.35 ml phosphate buffer (0.04 *M*; pH 7.4) in the presence of the NADPH regenerating system and after addition of $1\times10^{-4}$ *M* DOC as substrate. NADPH regenerating system: $0.74\times10^{-2}$ *M* potassium fumarate; $0.74\times10^{-2}$ *M* nicotinamide; $1.85\times10^{-3}$ *M* glucose-6-phosphate; $3\times10^{-3}$ *M* $MgCl_2$; $9\times10^{-4}$ *M* NADP. Chloroform extraction of the incubates. The corticosterone content was determined fluorometrically[9].

3. Steroid C21 hydroxylase. Microsomal suspensions from adrenals of freshly killed calves were used. The enzyme activity was measured by a colour reaction indicating the oxydation of 17$\alpha$-hydroxy-progesterone to 11 deoxycortisole[8,10,11]. The incubation medium was albumin glycylglycine buffer (pH 7.4) which contained the following: $3.3\times10^{-3}$ *M* glucose-6-phosphate; $0.6\times10^{-3}$ *M* NADP; 0.03 µl glucose-6-phosphate dehydrogenase. $2\times10^{-4}$ *M* 17$\alpha$-hydroxy-progesterone was used as substrate.

*Drugs and reagents.* 1 (+) ascorbic acid, Merck, No. 127 p.a.; cysteamine hydrochloride, Merck, No. 2835; cystamine dihydrochloride, Merck, No. 2834; 1 (+) cysteine hydrochloride, Merck, No. 2839; 2-aminoethanol (ethanolamin) Schuchardt, No. ME 013; 2-mercaptoethanol, Schuchardt, No. AM 031; 1.2-ethandithiol, Schuchardt, No. AE 012; 2.3-dimercapto-1-propanol, Schuchardt, No. DL 426. Glucose-6-phosphate dehydrogenase, Boehringer suspension, No. 15303. Metyrapone, Ciba-Geigy AG., Basel. The drugs were added to the incubation medium in a final concentration ranging from $0.4\times10^{-4}$ *M* to $4\times10^{-3}$ *M*.

The results are given as mean value and standard deviation. Student's *t*-test.

*Experimental results and conclusion.* 1. The inhibitory effect of cysteamine on the C11 $\beta$-hydroxylase was slightly weaker than that of metyrapone but in the same order of magnitude (Table I).

2. Data of the literature were confirmed[6,8,10] according to which the C21 hydroxylase was inhibited by ascorbic acid but not by metyrapone. ($0.1\times10^{-4}$ *M* to $1.0\times10^{-3}$ *M*). Cysteamine also did not affect the C21 hydroxylase, thus demonstrating anew the similarity of its influence on cortico-steroidogenesis to metyrapone.

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3. The comparison of cysteamine with the structure analogues 2-aminoethanol and mercaptoethanol showed that the inhibiting effect was lost when either the sulfhydryl (SH) group or the amino ($NH_2$) group was replaced by a hydroxyl (OH) group (Table II, No. 1, 2 and 3). From these results one must conclude that the inhibition by cysteamine requires the common presence of the SH and $NH_2$ group. This interpretation is also supported by the strong inhibiting effect of cystamine (No. 4) though this substance has no free SH group. However, the disulfide group of cystamine becomes rapidly reduced in vivo[12,13] and, in the presence of living tissue and cells, also in vitro[14].

These findings are in correspondence with the conjecture[2] that the inhibition by cysteamine of the 11 $\beta$-hydroxylase is caused by the formation of mixed disulfides. According to ELDJARN and PIHL[15], only the radioprotective thiols and disulfides are able to form mixed disulfides. From this point of view, the corresponding behaviour of cysteamine and cystamine in radioprotection (for ref. see BACQ[16]) and 11 $\beta$-hydroxylase inhibition is remarkable. The suggested role of mixed disulfide formation for the inhibition of the 11 $\beta$-hydroxylase is supported by the inactivity of cysteine (Table II, No. 5) that is unable to form mixed disulfides and to exert substantial radioprotection in vivo.

Unlike substances with one SH group, substances carrying two SH groups showed a significant 11 $\beta$-hydroxylase inhibition (Table II, No. 6 and 7). However, the effect of these substances was much weaker than that of cysteamine and cystamine. This weakness in hydroxylase inhibition is paralleled by an inability to form mixed disulfides and to exert radioprotection in animals[16].

From these results it is concluded that the mechanisms of the 11 $\beta$-hydroxylase inhibition exerted by metyrapone, and by mixed disulfide-forming thiols and by di-thiols, differ from each other in various ways.

*Zusammenfassung.* Cysteamine hemmt die Steroid 11 $\beta$-Hydroxylase schwächer als Metyrapon, jedoch stärker als Dithiole. Der Cysteamineffekt ist an die Konfiguration –SH–NH– gebunden, welche die Bildung gemischter Disulfide ermöglicht. Es werden Unterschiede im molekularen Mechanismus der Hemmwirkung zwischen den drei Stoffgruppen angenommen.

K. FLEMMING and VERA SEYDEWITZ[17]

*Institut für Biophysik und Strahlenbiologie der Universität, Abteilung Pharmakologie und Strahlenbiologie, Albertstrasse 23, D-7800 Freiburg (Federal Republic of Germany),*
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## Effet cumulatif de plusieurs anions sur une activité ATPasique $Mg^{2+}$-dépendante, de la fraction microsomale des pléopodes de *Sphaeroma serratum* (Fabricius)

Le système d'osmorégulation de *Sphaeroma serratum* permet à ce crustacé isopode de maintenir sensiblement constante la composition ionique de son hémolymphe malgré les variations importantes de la salinité du milieu environnant.

Des études antérieures[1] ont montré que les membranes plasmiques des cellules constitutives du tissu épithélial des pléopodes séparant le milieu intérieur du milieu ambiant contenaient une forte activité ATPasique $Na^+ - K^+$ dépendante, avec deux affinités distinctes pour chacun de ces ions. Un modèle de régulation des ions $Na^+$ et $K^+$ a été ainsi proposé faisant intervenir 2 pompes à sodium chacune sous la dépendance d'une ATPase-($Na^+ - K^+$).

Le fait qu'il existe un transport actif d'anions dans des tissus épithéliaux comparables à celui des endopodites comme les branchies de poisson euryhalin[2] et une ATPase stimulée par les anions dans d'autres tissus épithéliaux également impliqués dans des processus de secrétion ionique comme la muqueuse gastrique[3] ou pancréatique[4], nous a incité à chercher si la régulation du contenu ionique de l'hémolymphe n'était pas également sous le contrôle d'une pompe à anion elle-mème dépendante d'une ATPase spécifique. Les caractéristiques d'une telle enzyme sont présentées ici.

*Techniques.* Les pléopodes sont prélevés et homogénéisés dans un tampon histidine-imidazole $5 \times 10^{-4} M$ à pH 7,4. Lorsque cela est nécessaire, l'homogénat est divisé en ses différentes fractions subcellulaires par centrifugation différentielle qui sont caractérisées ultérieurement par le dosage d'enzymes spécifiques de ces fractions: ATPase ($Na^+ - K^+$) pour les membranes plasmiques, cytochrome c oxydase pour les mitochondries (technique de LEIGHTON et al.[5]).

L'activité de l'ATPase – $Mg^{2+}$ et de l'ATPase stimulée par les anions sont mesurées simultanément dans un milieu contenant 20 à 40 µg protéine/ml, 2 m$M$ Mg ($CH_3COO_2$), 2 m$M$ ATP, 60 m$M$ de tampon *tris* glycyl-glycine à pH 8,0, $10^{-4}$ g ouabaine/ml 0.5 m$M$ EDTA en présence ou non de 20 m$M$ de l'anion considéré. Après 30 min à 35°C la réaction est stoppée par addition d'acide trichloracétique glacé à 10% et le phosphate inorganique libéré est dosé par une technique voisine de celle de FISKE et SUBBAROW[6]. L'activité de l'ATPase sensible aux anions

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