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SHORT COMMUNICATIONS

Meclofenoxate decreases protein and albumin synthesis in hepatocytes isolated from old rats

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Meclofenoxate is the dimethylaminoethanol ester of the plant growth hormone parachlorophenoxyacetic acid. This ester is also known as centrophenoxine, clofenoxine, Helfergin® and Lucidril® and is used clinically in geriatric patients with symptoms such as confusion and memory disturbances. Activities described for meclofenoxate include: (1) an increase in the transport of glucose across the blood–brain barrier [1]; (2) stimulation of oxygen consumption of the brain [1]; (3) prevention of the deposition of the age pigment (lipofuscin) in postmitotic neurons [2]; and (4) an increase in the mean and maximal survival time of male mice [3, 4], but there is some controversy concerning these activities. For example, Tomonaga *et al.* [5] did not observe a decrease in lipofuscin accumulation in the brain after meclofenoxate treatment and the reported increase in the life-span could not be confirmed for aged Japanese quails [6].

Recent reports indicate that meclofenoxate might exert its action by stimulating the protein synthesis capacity of the brain [5, 7] and liver [8] of aged organisms. However, no direct proof was provided for an increase in the protein-synthesizing capacity of postmitotic cells in late age resulting from meclofenoxate treatment.

One of the objectives of this study was to investigate whether meclofenoxate treatment stimulated protein synthesis in postmitotic cells of aged rats. Suspensions of hepatocytes isolated from animals of different ages were used to study the action of meclofenoxate on postmitotic cells, especially with respect to a possible influence on the protein-synthesizing capacity.

Besides the establishment of its possible action on protein synthesis, it is of even more importance to know whether meclofenoxate can enhance the functional capacity of postmitotic cells in aged individuals. This question could also be studied with the isolated hepatocytes. Albumin synthesis and bromsulfophthalein (BSP) storage were chosen as liver-specific functions of the hepatocytes. We compared the protein and albumin-synthesizing and the BSP storage capacities of hepatocytes isolated from female WAG/Rij rats of various ages after meclofenoxate treatment with those of hepatocytes isolated from control rats of the same strain, sex and age group.

Materials and methods

Inbred virgin female WAG/Rij rats (a Wistar-derived strain) were used. The rats were maintained under 'clean conventional' conditions [9]. The hepatocytes were isolated by perfusion and incubation of the liver with the enzyme collagenase as reported earlier [10]. Cell concentrations were determined as previously described [10]. The experimental rats were injected intraperitoneally with 80 mg meclofenoxate/day per kg body weight for 2 weeks. Some animals in the group of rats of 31 months of age were also treated for 2 months, with treatment starting at the age of 29 months. Hepatocytes isolated from control and treated rats did not differ significantly in average yield (40×10^6 cells/g liver) and viability (at least 85% of the cells excluded trypan blue).

The protein-synthesizing capacity was determined from the incorporation of [^{14}C]leucine under optimum incubation conditions [10]. These conditions included the use of an enriched medium (Waymouth MB 752/1), cell suspensions ranging from 0.25 to 4×10^6 cells/ml of medium and incubation with a concentration of 6 μmole leucine/ml of medium for 2 hr at 37° under an atmosphere of 95% O_2 and 5% CO_2 [10]. Changes in incorporation rate due to breakdown of newly synthesized proteins or to differences in the specific radioactivities of leucine in the intra- or extracellular leucine pools could be excluded [10, 11]. The albumin synthesis was assessed after incubation of the hepatocytes in the Waymouth medium under an oxygen tension of 30×10^3 Pa at pH 7.8 for 3 hr by means of a radial immunodiffusion technique [12]. The BSP storage was determined by incubating the hepatocytes in suspension with [^{35}S]BSP. To achieve optimum BSP storage, the hepatocytes were incubated with a dose of 30 nmole [^{35}S]BSP/ml medium for 15 min at 37° [13].

Results

The BSP storage capacity and protein and albumin synthesis were determined for hepatocytes isolated from 31-month-old rats treated with meclofenoxate for 2 weeks and for 2 months. The data are presented in Table 1 and show no significant differences in these functional capacities. Therefore, in further experiments the rats of other age groups were treated with meclofenoxate for 2 weeks.

The data for the protein-synthesizing capacity of hepatocytes isolated from 3- and 12-month-old rats after meclofenoxate treatment did not show differences compared with control rats (Table 1). However, hepatocytes isolated from 24-, 31- and 36-month-old treated rats synthesized much less protein than did hepatocytes of the control rats of the same age groups (Table 1).

To some extent, the albumin synthesis of the hepatocytes isolated from the treated rats changed in a similar way in comparison with the control rats as was observed for the protein synthesis. Hepatocytes isolated from treated rats of 3 and 12 months of age synthesized the same amount of albumin as the hepatocytes isolated from the control groups (Table 1). The albumin synthesis of hepatocytes isolated from 24-month-old treated rats was higher than that observed for the control rats (Table 1). As observed for protein synthesis, the albumin synthesis of the hepatocytes isolated from treated rats of 31 and 36 months of age was much less than that found for the hepatocytes from the control rats of the same age (Table 1).

The BSP storage capacity of the hepatocytes isolated from rats of various ages was not influenced by the meclofenoxate treatment (Table 1).

Since both the protein and albumin-synthesizing capacities were determined, it was possible to ascertain whether the changes observed for the albumin-synthesizing capacity with age after meclofenoxate treatment were specific for albumin or might apply to all types of proteins synthesized by the hepatocytes. The ratio between albumin and protein synthesis by hepatocytes isolated from control and treated rats is shown in Table 2. This ratio was not influenced by the meclofenoxate treatment during the first year of life, increased significantly at 24 months of age, was not influenced at 31 months, and decreased at 36 months.

Table 1. Influence of meclofenoxate on functions of hepatocytes isolated from rats of different ages

Characteristic	Age (months)	Control group*	Meclofenoxate*-treated group
Protein synthesis (nmoles leucine/hr 10^6 cells)	3	14.4 ± 2.7 (10)	12.6 ± 6.7 (7)
	12	9.8 ± 3.5 (6)	7.3 ± 2.1 (7)
	24	8.4 ± 2.7 (5)	3.7 ± 1.0 (6)†
	31	12.9 ± 5.6 (4)	3.8 ± 1.3 (5)†-2w
			4.1 ± 1.2 (4)†-2m
	36	14.7 ± 4.7 (7)	9.2 ± 4.4 (10)†
Albumin synthesis (μg albumin/hr 10^6 cells)	3	5.8 ± 2.7 (12)	5.1 ± 2.3 (7)
	12	3.7 ± 1.0 (8)	4.4 ± 2.2 (6)
	24	2.29 ± 0.80 (7)	3.7 ± 1.1 (6)†
	31	6.4 ± 1.4 (3)	1.02 ± 0.80(5)†-2w
			1.21 ± 0.48(5)†-2m
	36	9.8 ± 4.6 (7)	2.6 ± 1.0 (9)†
BSP storage (nmoles/10^6 cells)	3	11.9 ± 4.9 (11)	7.8 ± 2.4 (6)
	12	8.3 ± 1.4 (8)	9.3 ± 1.2 (7)
	24	7.4 ± 3.0 (7)	6.3 ± 1.7 (6)
	31		6.5 ± 3.5 (4)-2w
			6.9 ± 1.5 (5)-2m
	36	6.4 ± 3.1 (6)	6.2 ± 2.4 (9)

* Mean ± S.D.

† Value differs significantly ($P \leq 0.05$) from the control value of the same age group.

The number of different cell preparations is given in parentheses. The 31-month-old WAG/Rij rats were treated with meclofenoxate for 2 weeks (2w) and 2 months (2m). The data of the control rats concerning the effect of age on the protein [10] and albumin synthesis [12] and BSP storage capacity [15] have been previously reported.

Table 2. Ratio of albumin vs protein synthesis by isolated hepatocytes as a function of the age of control and treated rats

Age (months)	Ratio albumin vs protein synthesis	
	Control group*	Meclofenoxate-treated group*
3	0.40 ± 0.24 (11)	0.40 ± 0.28 (7)
12	0.38 ± 0.17 (7)	0.60 ± 0.35 (7)
24	0.27 ± 0.13 (6)	1.00 ± 0.40 (6)†
31	0.50 ± 0.24 (4)	0.27 ± 0.23 (5)
		0.30 ± 0.15 (5)
36	0.67 ± 0.38 (7)	0.28 ± 0.17 (10)†

The data are expressed as: $\frac{\mu g \text{ albumin/hr} \times 10^6 \text{ cells}}{\text{nmole leucine/hr} \times 10^6 \text{ cells}}$.

* Mean ± S.D.; the number of different cell preparations is given in parentheses.

† Value differs significantly ($P < 0.05$) from the control value of the same age group.

Discussion

Hepatocytes were used as a model system for studying the effect of meclofenoxate on the aging of postmitotic cells. Reasons for choosing hepatocytes are that, like other postmitotic cells, they live as long as the individual and accumulate lipofuscin [14]. In addition, hepatocytes can be isolated from rats of different ages and the protein and albumin synthesis as well as the BSP storage capacity can be quantified. We previously observed that the protein and albumin synthesis and BSP storage capacity of isolated hepatocytes drastically changed with age [10, 13, 15].

The results in this paper, with respect to the action of meclofenoxate, show that this drug influences protein synthesis in postmitotic cells. However, no increase in protein synthesis, as was expected on the basis of the observations that meclofenoxate treatment increased the amount of rough endoplasmic reticulum (RER) in brain cells [5] as well as the protein content of the liver [7] and the RNA synthesis in cultured fibroblasts [6], was observed. On the contrary, the results in Table 1 reveal that the protein synthesis of hepatocytes isolated from old rats treated with meclofenoxate was decreased.

Changes were also observed in the ratio between albumin and protein synthesis by the hepatocytes isolated from meclofenoxate-treated rats compared with control rats. A change in albumin synthesis without a concommittant change in protein synthesis of the same order is quite possible, since only 10% of the synthesized proteins in young rats consists of albumin.

It can be concluded that the influence of meclofenoxate treatment on albumin and protein synthesis and on the ratio of these two activities are strongly age-dependent. In cells isolated during the first year of life, no influence of meclofenoxate treatment on these characteristics was

observed. At 24 months of age, the albumin synthesis increased, but the protein synthesis was decreased by the treatment so that their ratio increased. At 31 and 36 months of age, both albumin and protein synthesis were decreased. The decrease in albumin synthesis at 36 months between the control and treated rats was greater than the decrease in protein synthesis. Consequently, the ratio albumin vs protein synthesis by the hepatocytes of the 36-month-old treated rats is smaller than that of the control rats.

Since the BSP storage capacity of old hepatocytes was unchanged and the albumin synthesis was even decreased by meclofenoxate treatment, the conclusion can be drawn that meclofenoxate at the doses and times of treatment studied does not stimulate the functional capacities of old hepatocytes.

Protein-synthesizing activity was studied in hepatocytes isolated from rats of various ages with or without treatment with 80 mg meclofenoxate/day per kg body weight for 2 weeks. The substance did not influence protein synthesis in hepatocytes isolated from 3- and 12-month-old rats, but drastically decreased the synthesis of protein by hepatocytes isolated from 24-, 31- and 36-month-old rats. A comparable effect was observed for the liver-specific function of albumin synthesis. The absence of any stimulating effect of meclofenoxate treatment was also observed for another liver-specific function, viz. the BSP storage capacity. Therefore, the conclusion can be drawn that meclofenoxate at the doses and times of treatment studied does not stimulate the functional capacities of old hepatocytes.

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Antioxidant properties of some chemicals vs their influence on cyclooxygenase and lipoxidase activities

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Stimulation of cyclooxygenase by a number of chemical compounds has been reported [1–6]. This stimulation is well expressed in the absence of hydroquinone as a cofactor in the enzymic preparation [3] and requires high concentration of the substrate [6]. In the presence of hydroquinone [3] and at low concentration of the substrate [6, 7] the stimulators may act as inhibitors of cyclooxygenase. A suggestion has been put forward that stimulators of cyclooxygenase are scavangers of free radicals [6]. Indeed, antioxidants are supposed to act as cofactors for cyclooxygenase [8]. Also aminopyrine—a stimulator of cyclooxygenase [3]—was reported to be an antioxidant [9].

In this study we compare antioxidant effects of several chemicals with their influence on cyclooxygenase activity.

The compounds used were paracetamol (Galena, Poland), chlorpromazine (Polfa, Poland), eicosa-tetraynoic acid (TYA) (Hoffman La Roche, Switzerland), caffeic acid (Fluka, Switzerland), phenylhydrazine (Poch, Poland), compound BW 755 (Wellcome Research Laboratories, U.K.), compound KD 785 (Chinoin Laboratories, Hungary), aspirin (Polfa, Poland) and indomethacin (Polfa, Poland).

The effect of investigated compounds on non-enzymic and enzymic lipid peroxidation was assessed according to the principles previously described [9]. Non-enzymic lipid peroxidation was assayed as the amount of malondialdehyde (MDA) that had been formed during incubation of boiled rat liver microsomes in the presence of ascorbic acid after subtraction of the amount of MDA formed during incubation of boiled microsomes without ascorbic acid. Enzymic lipid peroxidation was measured as the amount of MDA formed during incubation of lyophilized native rat liver microsomes in the presence of NADPH after subtraction of the amount of MDA that had been formed during incubation of boiled microsomes with NADPH. The incubation mixture contained 0.8 ml of microsomal suspension (equivalent to 1 mg of protein), 0.1 ml of ascorbic acid solution (non-enzymic lipid peroxidation) or NADPH (enzymic lipid peroxidation), and 0.1 ml of aqueous solution of a tested compound or 0.1 ml of water. The final concentration of both ascorbic acid and NADPH was 200 μM. Samples were incubated for 90 min (non-enzymic lipid peroxidation) or 60 min (enzymic lipid peroxidation) at 37°. The oxidation of lipids was stopped by the addition