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STEREOLOGICAL ANALYSIS OF RAT ENDOCRINE PANCREAS AFTER CHRONIC ALCOHOL FEEDING

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A stereological study was carried out to analyse pancreatic endocrine A-, B-, and D-cells (immunostained by the PAP procedure) in 10 rats chronically consuming ethanol and 10 rats fed an isoenergetic standard diet for 4 months. The entire area of parenchymal tissue, including islets, was measured using a point-counting procedure. All islet profiles within the section were counted; their profile area, volume and numerical density, and the total volume were determined by point-counting, and their major and minor axes were measured. Morphometric measurement of the total number of A-, B-, and D-cells per islet and per μm^2 of islet, as well as volume and surface density of cell and nucleus were made. A standard stereological equation were used to calculate the above parameters. The mean diameter of the blood vessels was calculated from the formula $\overline{D} = 6Vv/Sv$, and their mean length from the formula $L = 4Vv/\pi\overline{D}$. The results obtained showed that chronic alcohol feeding significantly decreased the total volume of islets; the surface area, diameter and total volume of A-cells; all parameters investigated for B-cells, and surface area of D-cells. The volume fraction of islet blood vessels was significantly increased, as well as the diameter and length of islet blood vessels.

Key words: alcohol, endocrine pancreas, rat, stereology

INTRODUCTION

Although controversy regarding the definition and different histological types of alcoholic chronic pancreatitis in human and animal models prevails in the literature (Sarles, 1975; Singh 1987; Singer et al., 1990; De Angelis et al., 1992). the association between ethanol and chronic pancreatitis is well established. Functional studies showed that exocrine deficiency has a significant effect on islet cell function, and there are exocrine pancreas- insulin, glucagon, pancreatic polypeptide and somatostatin interactions, as well as

enteropancreatic interaction in chronic pancreatitis (Klpel et al., 1978; Go, 1990; reviewed by Owiang et al., 1991).

Because little is known about the effect of alcohol on islet endocrine cells, the purpose of this study was to determine the special attention to A-, B- and D-cells.

MATERIAL AND METHODS

Twenty male Wistar rats 2 months old, and weighing approximately 240 g, were randomly allocated into control (C) and ethanol (E) groups. Control rats were fed a commercial stock cereal based pelleted diet (25% protein). The ethanol group was given free access to a hyperprotein cereal based pelleted diet (34% protein) and an aqueous solution of 25% sucrose-32% athanol as recommended by Hartroft (1971). The amount of food fed to the control animals was adjusted to the energy intake of the animals receiving alcohol (i. e. the control rats were pair-fed). Ethanol was replaced by starch in the control diet. The experiment lasted for 4 months. At the end of the feeding period the animals were fasted overnight and the abdomen opened under light ether anaesthesia.

The pancreas was quickly removed and weighed in air. The total volume of the pancreas was determined using an immersion method (Scherle, 1970). Each pancreas was cut into 5 approximately equal parts and fixed in Bouin solution, embedded in paraffin, sectioned (5μm) and stained with Victoria trichrome stain (Kikui et al., 1976) and by immunohistochemical procedures. For analysis, 10 sections from the middle area of the tissue (2 per slide) were chosen from each region, totalling 20 sections per animal. Staining was performed in the following manner: the first slide was stained by the trichrome Victoria method, the second for neuron specific enolease (NSE); the third, fourth and fifth for insulin, glucagon and somatostatin, respectively. Immunohistochemistry was performed using a prediluted polycional antibody against neuron specific enolase (NSE; Zymed, San Francisco, CA) and polyclonal antibodies against insulin, glucagon (DAKO Corp., CA) and somatostatin (by courtesy of Dr J. Rehfield, Univ. Aarhus, Denmark). For NSE determination, sections were immunostained by the streptavidin biotin technique and for insulin, glucagon and somatostatin by the unlabelled peroxidase-antiperoxidase complex (PAP) technique.

Weibel's multipurpose test grid (M_{42}) was used to estimate volume (Vv) and surface (Sv) density of islet cells and islet blood vessels (Weibel et al., 1966). Numerical density of the islets was calculated from the formula $N_V=N_{A/D}$, where N_A was estimated from N/A_t. The mean islet diameter was determined directly on the microscope with the eyepiece micrometer. The mean surface area of islets and cells was obtained by point-counting using the equation A= ptx V3/2x d2. The volume of the average A-, B- and D-cell was estimated using the number of A-, B- and D- cell nuclei per μm^3 of A-, B- and D-cell mass (Loud, 1968). The mean diameter of the blood vessels was calculated from the formula D= 6Vv/Sv (Kališnik, 1985), and their mean length from the formula $L = 4Vv/\pi\overline{D}$. (Aherne and Dunnill, 1982).

The Student t-test was used to determine statistically significant differences.

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RESULTS

Table 1 summarizes some stereological parameters obtained for the islets of Langerhans in the control and experiment algroups of rats. It was found that all parameters investigated were slightly decreased in ethanol treated rats, except for the mean islet diameter. However, there were no statistically significant differences between the two groups of rats except that ethanol treated rats were found to have a significantly reduced total pancreatic islet volume. This reduction accounted for about 34% of the total islet volume in comparison with controls ($2p<0.05$).

Table 1. Stereological results for the islets of Langerhans in control (C) and experimental (E) rats

	C (n=10)	E (n=10)	2p
Volume density (mm^0)	0.006±0.012*	0.005±0.006	n.s.
Absolute volume (cm^3)	0.009±0.0010	0.006±0.009	0.05
Numerical density (cm^{-3})	9804±1414	8403±1456	n.s.
Absolute number	12378±1959	10640±2117	n.s.
Diameter (μm)	95.3±716	98.8±8.26	n.s.
Surface area (μm^2)	5360±923	5139±739	n.s.

*Mean SEM

The total number of endocrine cells both per islet profile and per μm^2 of islet (Table 2) was somewhat increased in alcoholic rats. This increase was probably due to an increase in the number of A- and D-cells. The number of B-cells, both per islet and per μm^2 of islet was decreased. Statistically significant differences were not found.

Table 2. Number of A, B and D-cells per islet profile and per μm^2 of islet

	C (n=10)	E (n=10)	2p
	Number/islet profile		
Total	55.8±2.82*	60.3±5.56	n.s.
A-cell	21.2±1.95	27.6±3.54	n.s.
B-cell	30.8±1.49	28.4±3.88	n.s.
D-cell	3.8±0.38	4.3±0.59	n.s.
	Number/μm2 of islet		
Total	0.01259±0.00167	0.01368±0.00193	n.s.
A-cell	0.00480±0.00087	0.00600±0.00103	n.s.
B-cell	0.00700±0.00086	0.00680±0.00113	n.s.
D-cell	0.00076±0.00010	0.00092±00013	n.s.

*Mean SEM

Morphometric results of A-, B- and D-cells are presented in Table 3.

As shown in Table 3, all endocrine cells in the rat islets of Langerhans were reduced in size, which indicated that ethanol had a strong effect on the cytoplasmic component of the cells. Besides that, the B-cell nuclei were found to be decreased in all the parameters investigated.

Table 3. Summary of morphometric results for the A, B and D-cells

	C (n=10)	E (n=10)	2p
	A-cell		
DIAMETER			
Cell	11.40±0.0.191*	10.16±0.315	0.01
Nuclei	6.65±0.11	6.52±0.06	n.s.
SURFACE AREA (μm^2)			
Cell	102.40±3.42	82.00±5.17	0.01
Nuclei	34.82±1.21	33.40±0.60	n.s.
VOLUME DENSITY (mm^0)			
Cell	0.24±0.026	0.27±0.022	n.s.
VOLUME (μm^3)			
Cell	777.25±38.76	563.46±53.77	0.01
Nuclei	154.24±8.03	144.51±3.87	n.s.
	B-cell		
DIAMETER (μm)			
Cell	12.16±0.176	11.24±0.192	0.01
Nuclei	7.29±0.12	6.50±0.19	0.01
SURFACE AREA (μm^2)			
Cell	116.40±3.41	99.50±3.41	0.01
Nuclei	42.28±1.55	33.56±1.71	0.01
VOLUME DENSITY (μm^0)			
Cell	0.70±0.028	0.66±0.024	n.s.
VOLUME (μm^3)			
Cell	940.31±41.89	745.07±38.39	0.01
Nuclei	203.36±9.74	147.18±9.88	0.01
	D-cell		
DIAMETER (μm).			
Cell	12.54±024	11.97±0.20	n.s.
Nuclei	7.02±0.10	6.92±0.08	n.s.
SURFACE AREA (μm^2)			
Cell	70.32±1.76	61.82±1.06	0.01
Nuclei	33.46±0.74	31.84±0.51	n.s.
VOLUME (μm^3)			
Cell	972.80±102.0	842.70±71.59	n.s.
Nuclei	170.70±12.5	172.70±7.6	n.s.

* Mean SEM

Histological examination revealed that islet blood vessels were dilated in alcoholic rats. This was proved by stereological analysis (Table 4). In ethanol-treated rats the volume density, diameter and total length of islet blood vessels were increased. A statistically significant difference was found for volume density ($2p<0.001$) and for total length of blood vessels ($2p<0.001$).

Table 4. Stereological results for blood vessels in the islets of Langerhans in the control (C) and experimental (E) rats

	C (n=10)	E (n=10)	2p
Volume density (cm^0)	0.025±0.003*	0.074±0.007	0.001
Diameter (μm)	9.73±1.23	12.62±1.05	n.s.
Total length (cm/islet)	0.003±0.0001	0.008±0.0001	0.001

* Mean SEM

Our results obtained for the rat pancreas after 4 months of alcohol ingestion revealed that there was no severe morphological injury of either the exocrine or endocrine pancreas. Klöppel et al,(1978) observed that a gross alteration of the endocrine pancreas existed if gross morphological disturbances of exocrine pancreatic tissue persisted, and viceversa. However, using stereological analysis, the main alteration in the endocrine pancreas was found to concern the total islet volume, size of endocrine cells and islet blood vessels.

Reduction of the total volume of the endocrine pancreas could be the result of a reduction in size of all endocrine cells, especially of the B-cells, indicating that ethanol somehow inhibits the function of the endocrine cells which causes their diminished size. An earlier report (Hellman and Hellerström, 1959) on the B-cell clearly suggested that the size of the B-cell was well correlated with their activity and function. Our results confirm earlier literature data that B-cells are more sensitive than the other islet endocrine cells to various experimental conditions or diseases (Koko et al., 1992).

Considering the anatomical position and angioarchitecture of islet cells and vessels (Bonner-Weir and Orci, 1982) it is possible that peptidergic activity in the pancreas caused that alteration. Recent studies on alcoholic chronic pancreatitis revealed gross disturbances to the peptidergic nerves in the pancreas. Their interactive effect probably resulted in inhibition of islet endocrine cells, especially B-cells (Büchler et al., 1992; Weihe et al., 1992).

The results obtained for the islet blood vessels (dilatation and changes in length) could be explained by the direct effect of ethanol on small blood arterioes. It has been reported that ethanol caused vasodilatation of small gastric arteriolar vesstriction of venules (Oates and Hakkinen, 1988) and this is in accordance with our results because most islets of Langerhans possessed small arterial blood vessels (Weaver and Sorenson, 1989).

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STEREOLOŠKA ANALIZA ENDOKRINOG PANKREASA PACOVA NAKON HRONIČNOG UNOSA ETANOLA

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SADRŽAJ

U deset pacova na četvoromesečnom alkoholnom tretmanu i 10 kontrolnih pacova sprovedena je stereološka analiza endokrinih ćelija pankreasa-A, B i D. Citohemijska identifikacija endokrinih ćelija izvršena je primenom imunohistohemijske PAP tehnike uz upotrebu poliklonskih antitela za insulin, glukagon i somatostatin. Površina parenhimskog tkiva, uključujući ostrvca, merena je point-kaunting tehnikom. Određivani su sledeći stereološki parametri za Langerhansova ostrvca: površina profila, volumenska i numerička gustina, ukupni volumen i maksimalni i minimalni dijametar. Morfometrijska analiza endokrinih ćelija podrazumevala je određivanje ukupnog broja A, B i D ćelija ostrvcetu i mm^2 površine ostrvceta, kao i volumensku i površinsku gustinu ćelija i nukleusa. Takođe su određivani i prosečna dužina i dijametar krvnih sudova ostrvaca. Rezultati pokazuju da hronično konzumiranje alkohola smanjuje ukupni volumen ostrvaca, površinu profila, dijametar i ukupni volumen A ćelija, kao i površinu profila D ćelija. Svi ispitivani parametri bili su redukovani u B ćelijama pacova na hroničnom alkoholnom tretmanu. Kod eksperimentalnih životinja registrovano je značajno povećanje volumenske frakcije, dužine i dijametra krvnih sudova.