Biochemical parameters of the histamine (HA) system were examined in both rat brain and stomach, after portocaval anastomosis (PCA). These tissues become rich in histamine after PCA. Immunocytochemistry was used for brain histamine localisation. In addition to increased HA concentrations, monoamine oxidase B activity increased in both tissues. In hypothalamus HA was 15 fold; in cerebral cortex and in stomach mucosa 2.8 and 2.5 fold of the corresponding controls, respectively. MAO B activity was increased by approximately 50% in brain and 100% in stomach. A significant, uneven increase in tele-methylhistamine concentration was only found in the brain. In stomach mucosa higher histidine decarboxylase activity was found.

PCA and sham rats treated with an irreversible inhibitor of MAO B, FA-73, 0.5 mg/kg i.p., showed 24 h later greatly reduced MAO activity and doubled t-MeHA concentration in brain structures. The treatment had no effect on gastric mucosal t-MeHA concentration and on urinary excretion of the t-MeHA metabolite, N-tele-methylimidazoleacetic acid. The HA rise in the stomach of PCA rats is associated with proliferation of histamine producing and storing cells (ECL cells) as demonstrated by others. However, in the brain we saw no indication for increased number of relevant cells either mast cells or neurons and our immunocytochemical findings suggest that in PCA rat brain, histamine deposits are located exclusively in neurons. The data indicate that the adaptative mechanisms to excessive histamine formation are tissue specific.

Key words: portocaval anastomosis, histamine system, brain, stomach mucosa, tele-methylhistamine

INTRODUCTION

Histamine (HA) functions as a potent stimulant of acid secretion in the stomach (1) and neurotransmitter/modulator in the brain (2). A local alteration in histamine synthesis and content was suggested in the 1960s as a primary cause of disturbed gastric functions in subjects with portocaval anastomosis (PCA). Experimental studies with portocavally shunted rats have shown that
higher gastric acid secretion in these animals coincided with a higher histidine decarboxylase (HDC) activity as well as a higher histamine concentration in stomach. Moreover, the acid hypersecretion could be attenuated by inhibiting HDC (3). Detailed histochemical, electron microscopical and biochemical studies (4) provided evidence that the changes in histamine system are brought about by an increased number, size and granularity of enterochromaffin-like (ECL) cells. ECL cells have capacity to synthesize, store and release histamine in response to feeding or injection of pentagastrin and insulin.

Portocavally shunted rats are a suitable animal model of hepatic encephalopathy (HE) (5) and in our studies on neurochemical alterations in the CNS triggered by chronic liver dysfunction we have found that an enhanced histamine synthesis occurred in brain in these animals (6, 7). Tissue histamine deposition is a predominant and unique mechanism of metabolic adaptation for cerebral amine neuromediators. Some lines of evidence indicate that the cells which accumulate histamine are neurons (6, 8) but this remains to be proven.

In brain that lacks diamine oxidase released histamine is metabolised via the methylation pathway (2). The same holds true for stomach (9). Activation of the methylation route in the two organs in PCA rats is strongly supported by significantly higher cerebral concentration of the intermediate metabolite, tele-methylhistamine (t-MeHA), as well as higher urinary output of histamine end product, N-tele-methyimidazoleacetic acid (t-MeImAA), of which major part is of a gastric origin (10). Here we attempted to gain further insight 1/ into regulation of HA metabolism in both brain and stomach by using FA-73 in vivo (11), a selective, more potent than deprenyl, irreversible inhibitor of monoamine oxidase B, for which tele-methylhistamine is an endogenous substrate (12), and 2/ into cellular location of brain histamine by employing HA immunostaining.

MATERIAL AND METHODS

All procedures strictly followed the Polish legislation concerning animal experiments and were approved by the local animal ethics committee.

Adult male rats of two strains Wistar and Lewis (body weight 250—300g) were used.

Surgery

End-to-side portocaval anastomosis was performed according to the method of Lee and Fisher (13). Sham-operated animals underwent laparotomy and occlusion of the portal vein for 10 min. Following surgery, rats were housed in wire mesh cages with a 12h light/dark cycle, light off at 7 p.m., and had free access to food and water.
Treatment

An irreversible inhibitor of MAO B, N-(2-propynyl)-2-(5-benzyloxyindol)methylamine, FA-73 (11) was a gift from Prof. M. Unzeta, Autonomous University of Barcelona, Spain, and was given as a single intraperitoneal injection in a dose of 0.5 mg/kg to Lewis rats of both PCA (n = 5) and sham (n = 5) groups, 6 months after surgery. The respective control counterparts (5 per group) received physiological saline (1.0 ml/kg i.p.).

Urine (24 h output) was collected between 48h—24 h before MAO inhibitor and again for 24 h after the drug was administered.

Urine collection was performed by placing rats individually in metabolic cages, where they had free access to tap water and food.

Sample preparation and biochemical analyses

Rats were sacrificed by guillotine decapitation. Weight of whole body and of some organs were recorded. From the brain hypothalamus and cerebral cortex were dissected. The stomach was opened along long curvature and cleaned in cold saline, blotted, weighed and the gastric mucosa was scrapped off. All tissue samples were immediately frozen in liquid nitrogen and kept at −70°C until assayed.

Cerebral histidine decarboxylase activity and histamine concentration were assayed radioenzymatically according to Taylor and Snyder (14) employing partially purified histamine N-methyltransferase from rat kidney (15). Gastric mucosa histidine decarboxylase activity was assayed with 1-carboxy-14C-L-histidine (16) whereas the tissue histamine concentration was determined by a fluorometric procedure after isolation on a small Cellex P column as described elsewhere (6).

MAO-A and MAO-B activities were estimated in tissue homogenates with radioassays (17) employing serotonin (final conc. 200 μM) or beta-phenylethylamine (final conc. 20 μM) and specific inhibitors deprenyl and clorgyline (0.3 μM each), respectively. N-tel-ethyl-methylhistamine was analysed by GC/MS as bis-heptafluorobutyryl derivative, with N-tel-D3-methylhistamine as an internal standard (18). Urinary N-tel-methylimidazoleacetic acid was converted to its isopropylester and determined by HPLC with UV detection; N-tel-ethyl-imidazoleacetic acid was an internal standard. With the same HPLC separation procedure urine creatinine concentration was determined enabling the expression of excreted t-MeImAA in both μg/24h and mmol/mmol creatinine (19).

Immunocytochemical study of histamine containing cells in the PCA rat brain

In situ brain perfusion with 4% 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in 0.1 M sodium phosphate buffer, pH 7.0, (PB) was performed
on anesthesized rats as described elsewhere (20, 21). The brains were removed, immersed in the same fixative overnight and thereafter in 20% sucrose in PB overnight. Cryosections (20 μm or 40 μm) were washed in phosphate buffered saline (PBS) containing 0.25% Triton X-100 (PBS-T) and incubated overnight with the rabbit anti-histamine antiserum 19C (22) diluted 1:1000 in PBS-T containing 1% normal goat serum. After a subsequent wash in PBS-T the 20 μm sections were incubated with FITC-conjugated swine anti-rabbit IgG (DAKO, Copenhagen, Denmark) diluted 1:40. They were then used for fluorescence microscopic studies. The 40 μm sections were treated similarly with the exception that the FITC-conjugated IgG was exchanged for Alexa 568 goat anti-rabbit IgG diluted 1:500 and the sections were used for confocal microscopic studies. A whole brain was stained according to the latter protocol with the exception that 1% DMSO was included in the primary incubation and that the incubations lasted two nights each and the final wash overnight. The sections and the whole brain were then rinsed with PBS and mounted with glycerol-saline (1:1) (1% DABCO was added to the whole brain mounting medium to decrease fading) and then examined with a LEICA TCS SP confocal microscope coupled to an ArKr-laser. The fluorophore was excited at 568 nm and fluorescence emission between 580 and 660 nm was recorded. The sections were scanned at several depths and maximum projection-images were made out of the data. The data were processed with LEICA TCS NT software.

The histamine antiserum used does not detect L-histidine or histidine-containing peptides as studied by dot-blot methods. Conjugates containing L-histidine did not abolish the staining. Preadsorption with a histamine-ovalbumin or histamine-BSA conjugate (1 μg/ml) abolished the immunofluorescence.

Statistical analysis

The values are presented as mean ± SD. Differences between groups were assessed with paired or unpaired t-tests as appropriate.

RESULTS

General effects of PCA

The data in Table 1 demonstrate the characteristic general effects of portocaval shunt. Six months after the surgery shunted rats had body weight about 35% lower than sham operated rats, even if their daily feed and fluid intake was relatively higher. The relative liver weight (i.e. ratio of liver weight
Table 1. General effects of portocaval shunt.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt, g</th>
<th>Daily feed consumption, g/100g body wt</th>
<th>Daily fluid intake, ml/100g body wt</th>
<th>Liver wt, g</th>
<th>Liver index (Liver wt/body wt x 100, %)</th>
<th>Stomach, g</th>
<th>Gastric mucosa, g</th>
<th>Mucosal index (Mucosa wt/stomach wt x100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA (n = 10)</td>
<td>247.0 ± 43*** p &lt; 0.0001</td>
<td>8.0 ± 1.7*** p = 0.0002</td>
<td>23.7 ± 7.7*** p &lt; 0.0001</td>
<td>4.61 ± 0.99*** p &lt; 0.0001</td>
<td>1.87 ± 0.22*** p = 0.0005</td>
<td>1.65 ± 0.16* p = 0.0208</td>
<td>0.434 ± 0.07* p = 0.0101</td>
<td>26.46 ± 3.74*** p = 0.0001</td>
</tr>
<tr>
<td>SHAM (n = 10)</td>
<td>382.0 ± 49</td>
<td>4.6 ± 1.3</td>
<td>7.9 ± 1.9</td>
<td>9.84 ± 1.55</td>
<td>2.82 ± 0.66</td>
<td>1.82 ± 0.15</td>
<td>0.344 ± 0.06</td>
<td>18.86 ± 2.92</td>
</tr>
</tbody>
</table>

Lewis rats were 6 months after portocaval shunt (PCA) or sham operation.

Table 2. Histamine system in brain and stomach. The effect of portocaval shunt.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Tissue</th>
<th>Group</th>
<th>HA, nmol/g</th>
<th>t-MeHA, nmol/g</th>
<th>HDC, pmol/min/mg protein</th>
<th>Enzyme activity, pmol/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MAO A</td>
</tr>
<tr>
<td>Brain</td>
<td>Hypothalamus</td>
<td>PCA</td>
<td>21.8 ± 7.2*** p &lt; 0.001</td>
<td>2.51 ± 0.65* p &lt; 0.05</td>
<td>0.97 ± 0.34</td>
<td>1267 ± 54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SHAM</td>
<td>1.44 ± 0.17</td>
<td>1.19 ± 0.70</td>
<td>1.11 ± 0.39</td>
<td>1202 ± 95</td>
</tr>
<tr>
<td></td>
<td>Cerebral cortex</td>
<td>PCA</td>
<td>0.84 ± 0.18*** p &lt; 0.001</td>
<td>0.54 ± 0.12* p &lt; 0.05</td>
<td>0.19 ± 0.05</td>
<td>1353 ± 75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SHAM</td>
<td>0.30 ± 0.08</td>
<td>0.37 ± 0.02</td>
<td>0.17 ± 0.03</td>
<td>1252 ± 107</td>
</tr>
<tr>
<td>Stomach</td>
<td>Mucosa</td>
<td>PCA</td>
<td>1787 ± 523*** p &lt; 0.001</td>
<td>1.25 ± 0.47</td>
<td>29.83 ± 13.64*** p = 0.001</td>
<td>692 ± 108</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SHAM</td>
<td>706 ± 168</td>
<td>1.24 ± 0.20</td>
<td>6.30 ± 1.77</td>
<td>604 ± 109</td>
</tr>
</tbody>
</table>

Lewis rats 6 months after portocaval shunt (PCA) or sham operation were examined. Values are means ± SD of n=5 rats per group. Histamine (HA) was assayed radioenzymatically (14) or spectrofluorometrically (7); tele-methylhistamine (t-MeHA) by GC/MS (18); Histidine decarboxylase (HDC) and monoamine oxidase (MAO A and MAO B) activities by currently used isotopic procedures (14, 16, 17).
to body weight \( \times 100\% \) was lower, however, the relative stomach weight was higher, in PCA versus sham rats. The former reflects atrophic changes that take place in the liver, the latter indicates that in poor nutritional state there are compensative changes in stomach as further evidenced by mucosa thickening (Tab. 1).

**Effects of PCA on HA system in brain and stomach**

Table 2 summarises the results describing histamine system parameters in brain (hypothalamus and cerebral cortex) and in gastric mucosa of the shunted rats, as opposed to sham operated ones. In both organs increased histamine concentrations and monoamine oxidase form B (MAO B) activity were found. In the hypothalamus HA was increased 15 fold; in cerebral cortex and in stomach mucosa the increase was less and similar: 2.8 and 2.5 fold, respectively. MAO B activity was increased by roughly 50% in brain but was doubled in stomach. There was also a significant increase in tele-methylhistamine concentration in brain, however, not as large as that of HA. In cerebral cortex the t-MeHA level was increased by half and in the hypothalamus slightly exceeded 2 fold. In turn, in stomach mucosa a high, almost 5 fold increase compared to the normal, histidine decarboxylase activity was found.

It is interesting to note, that the concentration of t-MeHA in gastric mucosa which was not changed upon shunting, closely resembles that found in hypothalamus (1.24 ± 0.20 vs 1.19 ± 0.70).

**Effect of MAO B inhibitor administration**

A single dose of FA-73 did not influence the daily feed or fluid intake by PCA or sham rats. As shown in Fig. 1A all rats treated with FA-73, and sacrificed 24h later, expressed greatly reduced cerebral MAO B activity (by 80—90%) and lower by about 30% the gastric mucosa enzyme activity (Fig.1 A). In these rats, brain t-MeHA concentration (Fig.1 B) was roughly 2 fold higher. Thus, in PCA rats with already increased cerebral t-MeHA (Tab. 2), monoamine oxidase B inhibition caused a further tissue amine increment, so their mean hypothalamic level was about 5 fold and cerebral cortex 3 fold of the corresponding tissue of untreated sham operated counterpart.

No difference in t-MeHA concentration in the gastric mucosa was noted either in PCA or sham groups.

The treatment also had no effect on t-MeHA metabolite, N-tele-methylimidazoleacetic acid urinary excretion (Fig. 1 C). However, in
Fig. 1. The effect of irreversible inhibitor MAO B (FA-73, 0.5 mg/kg i.p. – 24h) on panel A: monoamine oxidase B activity; panel B: tele-methylhistamine concentration in brain parts and stomach mucosa and panel C: urinary excretion of tele-methylimidazoleacetic acid by portocavally shunted (PCA) and sham operated (SHAM) rats 6 months after surgery. The means ± SD are given. The results in Fig. 1 A & B are expressed as % of untreated counterparts. For absolute values refer to Tab. 2.
agreement with the earlier study (10), the daily output of t-MeImAA by PCA rats was 3—5 fold that of sham rats.

It should be stressed that in the dose employed, the compound FA-73 exerted no effect on MAO A activity in any examined tissue. Likewise, it did not affect tissue histamine concentration or HDC activity.

Brain histamine location; non-neuronal versus neuronal compartment

Wistar rats, 4—9 weeks after the surgery were examined. There was no significant difference in HA-immunoreactivity between 4-week and 2-month PCA. The representative results of immunocytochemical study on cellular histamine location in brain are shown in Figs 2 and 3. Mast cells were not observed in any brain areas other than lateral thalamus and median eminence and following portocaval shunt neither their number was changed nor intensity of HA immunostaining was different (not shown). This was in contrast to the neuronal fibre histamine immunofluorescence in PCA rats, which was evidently more intense in several brain areas. On the other hand, the occurrence and density of histamine immunoreactive neurons and nerve fibres was essentially the same for PCA and sham animals (Figs 2, 3). In PCA rat

![Image of histamine-immunoreactivity in the PCA rat one month after operation and sham-operated rat brain. A) A view of a coronal section of the caudolateral hypothalamus of PCA rat shows several large varicosities. B) The corresponding sham-operated animal displays a fairly high density of normal histaminergic nerve. Scale bars = 100 μm.](image)
Fig. 3. Confocal microscopy maximum projection images of HA-stained hypothalamus of rats that have been subjected to portocaval shunt for four to nine weeks. A) An overview of the posterior hypothalamus in coronal plane. Two of the characteristic large cisternae are marked with arrows. B) A higher magnification image of an area corresponding to the lower right corner in A showing the morphology of three objects with high accumulations of HA-immunoreactivity and a few short fiber sections. C) A horizontal overview of the caudolateral hypothalamic surface shows numerous intensely HA-immunofluorescent large varicosities and one intensely HA-immunofluorescent neuronal cell body (arrow). Scale bars in A, C = 100 μm, B = 10 μm.
brain unusually large varicosities and cisternae intensely immunoreactive for histamine, formations that were never seen in control rat brain, were observed in hypothalamus (Fig. 2) and in other areas f.ex. amygdala, substantia nigra and cerebral cortex. The histamine immunofluorescence was elevated not only in the fibers. An elevation, although one not as obvious, could also be seen in the cell bodies of histaminergic neurons (Fig. 3) but the contribution of the fibers to the total histamine immunofluorescence was significantly higher in PCA rats as compared to normal ones. Confocal microscopy indicated that the histamine-containing cisternae were irregular in shape and size (Fig. 3).

**DISCUSSION**

We have confirmed here that portocaval shunt in the rat is associated with an enhanced histamine synthesis in brain and gastric mucosa resulting in the high tissue amine concentration. In brain it is related to better HDC saturation with L-histidine (6); in stomach it is due to ECL cell proliferation (4). Intensified HA synthesis raises the question concerning counteracting adaptational mechanism(s). Under normal conditions, in brain, histamine released from histaminergic nerve terminals or varicose fibers is methylated mainly in postsynaptic or extrasynaptic neurons (23, 24) although astrocytes also appear to contain histamine N-methyltransferase (25, 26). In stomach, released histamine is taken up and methylated by oxyntic cells (27).

GC/MS analysis revealed that in PCA rats the concentration of the methylation product, tele-methylhistamine, increases, only in brain and not in the stomach (Tab. 2).

In the brain tele-methylhistamine is an endogenous substrate of monoamine oxidase B (12, 28). In peripheral tissues, the common belief is that both, monoamine oxidase and diamine oxidase can degrade t-MeHA (9, 29). Interestingly, monoamine oxidase B activity was significantly higher in brain as well as in stomach in the portocavally shunted rats (Tab. 2), suggesting that the adaptive rise in the enzyme protein could be induced by the higher t-MeHA substrate supply. However, the suicidal MAO B inhibitor which was administered to test this concept, evoked an increase in tele-methylhistamine concentration only in brain. The effect was the same for the shunted and sham operated rats (Fig. 1B). It should be noted however that the inhibitory effect of FA-73 on MAO B activity was much higher for the brain than for stomach mucosa (Fig. 1A), the difference probably related to the enzyme turnover rate, which is much slower for the brain than for peripheral tissues (30). Thus, the remaining activity (ca 70% control) could still be high enough to maintain metabolism; the urinary excretion of the histamine end product, tele-methylimidazolecetic acid was not significantly affected by the treatment.
Alternatively, diamine oxidase (31) may be responsible for the oxidative deamination of t-MeHA to t-MeImAA. The concentration of tele-methylhistamine which was found in gastric mucosa in the rat closely resembles that in hypothalamus [1.24 nmol/g, Table 2] and it is of the same order of magnitude as in porcine gastric mucosa [2.5 nmol/g] (32) or in whole stomach in mouse [2.5-4 nmol/g] (33, 34). However, the ratios of t-MeHA to histamine in the same tissues (tMeHA = 1) show marked differences between species, i.e. 1:570 (rat) 1:60 (pig) 1:20 (mouse). In rat brain the corresponding ratio is almost 1:1. On this basis, it can be concluded that in rat stomach mucosa, in contrast to the brain, only a very tiny fraction of tele-methylhistamine is retained by the tissue and this might suggest very limited capacity/ability of the rat gastric mucosa to store t-MeHA. In other words, after being formed in oxyntic cell, t-MeHA leaves the stomach. Schippert et al. (32) in pig gastric mucosa did not detect any other histamine metabolite beyond t-MeHA. In a more recent study, it was shown that after pentagastrin infusion in dogs, histamine and tele-methylhistamine secretion could be followed in gastric venous plasma, suggesting that not all of the released HA is metabolised by gastric mucosa (35). These data imply that further metabolism of t-MeHA, unlike that of in the brain (36), takes place extragastrically. If therefore further metabolism continues at the level of intestine, diamine oxidase is more likely to exclusively participate in it (37, 38).

We have confirmed here the high potency of the compound FA-73 in inhibiting cerebral MAO B in vivo (11). Thus, FA-73 in this low dose of 0.5mg/kg ip gave a similar reduction of the enzyme activity as 3 mg/kg sc deprenyl (28). In this context, we feel obliged to add that the treatment with FA-73 did not affect daily feed consumption either by portocavally shunted or sham operated rats, although MAO B inhibition with deprenyl (5 mg/kg) has been reported to be associated with reduction of gastric mucosal blood flow and basal acid secretion in rat (39).

In the central nervous system histamine is present in neural and non-neural pool, the second comprises mast cells and microvascular endothelial cells (2). In portocavally shunted rats an increase in histamine concentration is invariably found in different brain areas, raising the obvious question which cell type stores the amine. Uneven and widely spread, this increase does not fit to mast cell compartment engagement (40) and indeed, biochemical approaches excluded the mast cell as the site and fully supported the concept of its neuronal location (6, 8). Likewise, in the immunocytochemical part of our present study we have found that mast cell number, location and their HA-immunoreactivity in PCA and sham brain are similar. Comparison of the images obtained for portocavally shunted and sham operated rat brain shows clearly that characteristic histaminergic innervation was totally preserved following shunting and the main difference was much higher intensity of
histamine immunoreactivity and the presence of large cisternal structures in PCA brains. In PCA rats varicose fibers expressed much more intense, bright fluorescence and pathologic formations with accumulated histamine and neuronal cell bodies often appeared somewhat brighter than in sham operated animals. All these findings firmly suggest that it is neuronal compartment which is enriched in histamine following portocaval shunt.

Portocaval shunt is associated with excessive synthesis of other amine neurotransmitters (5, 7) however, described histamine neuronal deposition is an unique countermechanism.


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REFERENCES


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