Discussion

FMLP has previously been detected in culture filtrates of *E. coli*[3]. In addition, these authors detected 4 other formyl peptides although the structures were not determined. We have confirmed the presence of FMLP in *E. coli* culture fluid (fig. 3a). In addition to FMLP, two other tripeptides (f-met-ser-leu, f-met-phe-leu), two tetrapeptides (f-met-met-ile-ala, f-met-gly-met-ile) and a hexapeptide (f-met-val-phe-ile-leu-leu) were demonstrated (table).

The structures presented may represent only some among many formyl peptides secreted by *E. coli*. Some fractions which elicited lysosomal enzyme release by human leucocytes did not generate sequential amino acid release on carboxypeptidase Y digestion. The concentrations of these putative peptides may be insufficient to yield detectable amino acids by OPA derivatisation. Alternatively, peptides containing amino acids which are not detectable by OPA derivatisation (e.g. cysteine and proline) may trigger the PMN response. However the structure/function specificity of the formyl peptide receptor suggest that this is probably not so[1].

There is accumulating evidence suggesting that low molecular weight pro-inflammatory N-formyl-methionyl oligopeptides could play a role in intestinal inflammatory disorders. Intestinal bacteria produce such peptides in vitro and bioactive peptides have been demonstrated in colonic fluid obtained by in vivo dialysis techniques[3]. In experimental animals both colonic infusion and rectal administration of millimolar concentrations of FMLP resulted in experimental colitis[7,8]. While healthy rat colon shows only very limited permeability to luminal formyl-peptides, experimental damage to the colonic mucosal barrier markedly increases peptide absorption[9]. Absorbed formyl-peptide is excreted by the liver into bile undergoing entero-hepatic circulation, providing a pathophysiological basis for the association of hepatobiliary and intestinal inflammation[9,10]. Determining the structures and properties of naturally occurring N-formyl oligopeptides is one aspect of defining their possible role in inflammatory disorders of the gut and extra-intestinal sites.

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In vivo degradation of noradrenaline by MAO A in locus coeruleus of the rat

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Summary. Depletion of noradrenaline in locus coeruleus neurons after reserpinization was prevented by clorgyline, a selective inhibitor of MAO A, but not by deprenyl, a selective inhibitor of MAO B. Only MAO A is therefore responsible for the degradation of homoneuronal noradrenaline in locus coeruleus nerve cells.
Key words. MAO A; MAO B; locus coeruleus; rat; noradrenaline histofluorescence; reserpine; deprenyl; clorgyline.

Different forms of MAO (monoamine oxidase – monoamine: oxygen oxidoreductase; EC 1.4.3.4) are present in the central nervous system. They are classified as mitochondrial enzymes MAO A and MAO B and the cytosolic MAO. The latter differs from MAO forms A and B by its resistance to inhibition by clorgyline and deprenyl[1–3]. In the present investigation, special attention was paid to the possible functional role of the above-mentioned forms of MAO in the noradrenergic neurons of the locus coeruleus (LC). The LC differs from other nuclei of the rat brain by showing the highest activity of MAO[4]. Intensively stained MAO-positive neurons were shown in the LC by an improved histochemical method[5]. Previous reports on the localization of molecular forms of MAO (A and B) in the LC were based on histochemical, immunohistochemical and microgasometric methods. Histochemical staining demonstrated the presence of MAO A in neurons and MAO B in the nerve endings

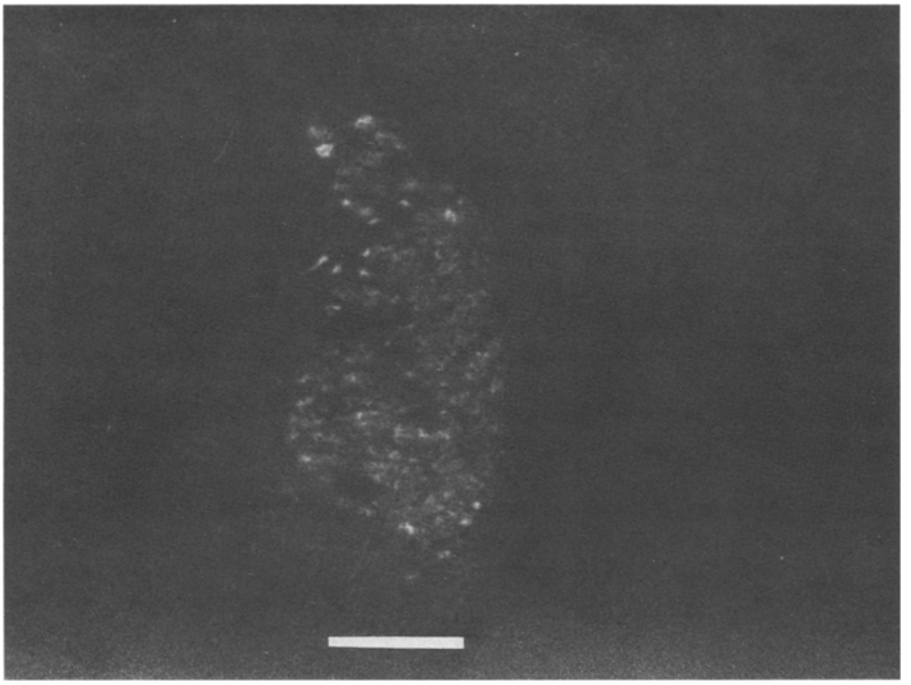

Figure 1. Glyoxylic acid-induced fluorescence of noradrenaline in nerve cells of locus coeruleus of a control rat. Bar 150 µm.

in the LC of the cat[6]; both forms were found in the LC of the mongolian gerbil[7]; only MAO A was present in the LC neurons in marmosets[8] and in rats[9]. Only MAO A was detected by the immunocytochemical method in human LC neurons[10].

The microgasometric method revealed the presence of two molecular forms of MAO in LC nerve bodies of the rat[11]. On the other hand, no data concerning the histochemical localization of the soluble, clorgyline- and deprenyl-resistant MAO in the brain is available. In order to assess the possible functional importance of the different forms of MAO for the metabolism of noradrenaline (NA) in LC neurons, the animals were treated with clorgyline (selective inhibitor of MAO A) and deprenyl (selective inhibitor of MAO B)[12]. The selective inhibitor that inactivates the functionally important form of MAO should prevent the depletion of NA from the LC neurons. The possible functional role of the soluble cytosolic MAO, which can be inhibited by neither of the two inhibitors, was indirectly assessed in the same experiments.

Methods and materials

Four groups of five animals (female Wistar rats, 170–240 g) were treated according to the following schedule:

a) first group: isotonic saline s.c., 30 min prior to sacrifice (controls);
b) second group: reserpine 10 mg/kg i.p., 30 min prior to sacrifice;
c) third group: clorgyline hydrochloride 2 mg/kg s.c., 4 h prior to sacrifice, in addition to reserpine as in b);
d) fourth group: l-deprenyl hydrochloride 10 mg/kg s.c., 4 h prior to sacrifice, in addition to reserpine as in b).

The dosages of reserpine and of the MAO inhibitors were chosen according to the data in the literature[13–15]. The animals were decapitated under ether anaesthesia. The brains were quickly dissected free, frozen in dry ice, transferred to the cryostat and cut into 20-µm sections at 253 K. The LC was localized by staining the slices with 0.5% methylene blue. Six slices from each LC were processed according to the glyoxylic acid method[16] and examined under the fluorescence microscope using epiillumination (Opton, mercury lamp, BG 12 excitation filter, 500 nm barrier filter). The intensity of green fluorescence of NA was estimated by two independent observers and graded semiquantitatively (strong fluorescence 3, moderate fluorescence 2, feeble fluorescence 1, no fluorescence 0; intermediate values 0.5, 1.5, 2.5), as well as photographed (5-min exposure, Ilford HP5 negative, fig. 1). The substances used were: reserpine (Serpasil amp. à 2.5 mg/ml, Ciba, Basel, Switzerland); clorgyline hydrochloride (May and Baker, Dagenham, UK); l-deprenyl hydrochloride (Chinoin, Budapest, Hungary); glyoxylic acid (Sigma, St. Louis, Mo, USA).

Results and discussion

The frequency distributions of the estimates of fluorescence intensities are presented (fig. 2a,b,c,d), one for each experimental group. The LC slices exhibiting no fluorescence (0) or traces of fluorescence (0.5) were considered as being depleted of NA; the LC exhibiting fluorescence levels 1–3 were defined as nondepleted.

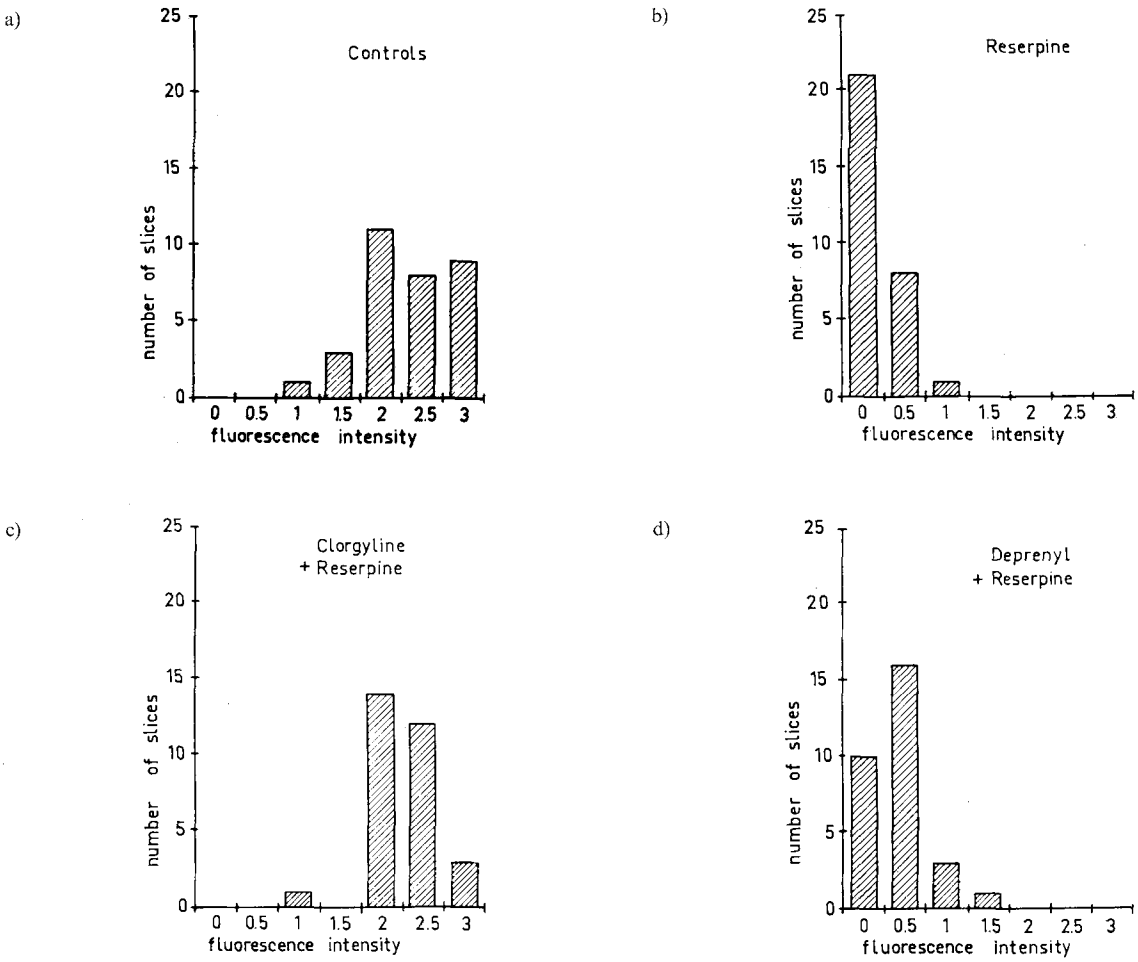

Figure 2. Frequency distribution of glyoxylic acid-induced fluorescence intensities in slices of locus coeruleus from rats: *a* controls; *b* injected with reserpine; *c* pretreated with clorgyline and injected with reserpine; *d* pretreated with deprenyl and injected with reserpine.

In the first group of rats (controls; treated by saline solution) moderate to strong fluorescence was observed (fig. 2a).

The second group was used to demonstrate the depletion of NA by reserpine. Reserpinization caused depletion of NA (traces of or no fluorescence) as shown in figure 2b.

In the third group of rats (initially treated with clorgyline to inhibit MAO A and then treated with reserpine) no depletion of NA was observed (fig. 2c).

The fourth group (initially treated with deprenyl to inhibit MAO B and later with reserpine) showed only a very feeble antagonistic effect on reserpine-induced depletion of NA histofluorescence (fig. 2d).

For the proper evaluation of our results, the degrees of inhibition of both molecular forms of MAO must be taken into account. The degree of inhibition of MAO A and MAO B in rat brains after a single parenteral dose of either clorgyline or deprenyl was determined biochemically by other investigators[14,15]. Clorgyline at a single dose of 2 mg/kg inhibited more than 90% of MAO A activity but less than 10% of MAO B activity. On the other hand, l-deprenyl in a single dose of 10 mg/kg inhibited about 90–97% of activity of the B form and 30–50% of the MAO A form. This modest but not negligible inhibitory effect of deprenyl on MAO A activity is probably responsible for the slight protective action of deprenyl on reserpine-induced depletion of NA, which was found. The effective prevention of reserpine-elicited depletion of NA in LC neurons by the MAO A inhibitor and the almost complete absence of prevention by the MAO B inhibitor clearly reveals an important functional role of MAO A for the intraneuronal metabolism of NA in the LC neurons of rats. The results also indirectly exclude the possible functional significance of the cytosolic MAO in this respect because this form of the enzyme is not inhibited by clorgyline[3]. If it was present in any significant amount, it should have degraded the NA released into the cytosol by the action of reserpine. Even in clorgyline-treated rats with inhibited MAO A, this was not the case.

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VIP receptors and control of short circuit current in the human intestinal clonal cell line Cl.19A

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Summary. At the maximally effective concentration of 10 nM, VIP induced a marked (12.5-fold stimulation above basal), and sustained increase in short circuit current in the human intestinal epithelial cell line Cl.19A grown on permeable filters and placed in Ussing chambers. Half-maximal increase of Isc was observed for 0.1 nM VIP. This was well correlated with the VIP-stimulated adenylate cyclase activity (ED_{50}: 0.07 nM). Binding studies using ^{125}I-VIP indicated that Cl.19A cells express a peptide-specific VIP receptor with a dissociation constant of 0.07 nM. Covalent labeling of receptors followed by SDS-PAGE analysis of membrane proteins resulted in the identification of a 63 000 dalton binding protein in Cl.19A cells.
Key words. Colonic epithelial cell line; VIP receptors; short circuit current.

The neuropeptide vasoactive intestinal peptide (VIP) plays a pivotal role in controlling cyclic AMP-dependent secretion of water and electrolytes in the intestine[1]. Several cultured cell lines derived from human colonic adenocarcinomas have been used either for documenting the mechanisms whereby VIP induces chloride secretion, or for examining some properties of VIP receptors[2–5]. However, it is difficult to correlate the results derived from these two types of studies since they have been conducted independently on different models.

Therefore we were prompted to explore within the same intestinal cell line the functional and molecular characteristics of VIP receptors, the ability of VIP to stimulate adenylate cyclase, and the effects of VIP on short circuit current in cells grown on permeable filters and mounted in Ussing chambers. The human intestinal Cl.19A cell line[6] was chosen as it had previously been shown to have the properties of a chloride secreting epithelium[7].

Materials and methods

Cell line: the Cl.19A[6] cell line is a differentiated clonal derivative of the human colonic adenocarcinoma cell line HT29[8]. The isolation as well as the culture characteristics of these cells have been described elsewhere[6]. Briefly, the Cl.19A cells have been shown to form, on reaching confluency, a typical monolayer with the apical cell surfaces separated from the basolateral surfaces by tight junctions.

The Cl.19A cells were routinely cultured in Dulbecco's modified Eagle's medium, plus 10% heat inactivated foetal bovine serum. The cells were routinely screened for mycoplasma contamination using Chen's method[9] and they were always found to be negative.

For electrical analysis of transport function, the cells were seeded at a density of 800,000 cells per cm^2 on collagen-coated permeable filters (diameter: 13 mm; HAHY millipore filters)[10]. On day 16 of culture, each filter was placed as a flat sheet in an Ussing chamber. Short circuit current was measured as previously described[2,3].

Membranes were prepared from post-confluent cells as described[10]. Adenylate cyclase activity was assayed following the previously published method[11]. The binding assay of ^{125}I-VIP was conducted as described[11]. The incubation medium contained in a 250-µl final volume,