IMPLICATION OF ACTIVE METABOLITES OF AMITRIPTYLINE AND NORTRIPTYLINE IN RAT PLASMA

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In previous studies amitriptyline and nortriptyline were given to depressive patients, human volunteers and rats, in order to establish the possible correlation between the drug concentration (assayed by gas chromatography) and the biological effect (indicated by noradrenaline or 5-HT uptake in brain synaptosomes or blood platelets). It was demonstrated that plasma concentration of tricyclics and the inhibitory activity of plasma on amine uptake correlated fairly well during the first hours after drug administration to volunteers. However, the biological effect tended to outlast the assayable drug concentrations. During long-term treatment the biological activity was remarkably higher than what could be anticipated on the basis of drug concentrations.

In the present study, 0, 1, 3 or 10 mg of amitriptyline or 0, 1, 3, 10 or 30 mg of nortriptyline were given i.p. to rats. The rats were bled after 1 hour, and plasma samples were used as incubation medium for intact rat synaptosomes as described previously (Tuomisto et al., 1980).

Uptake in plasma from amitriptyline-treated rats was lower than in control samples. Inhibition of both 5-HT and noradrenaline uptake was dose-dependent: at the dose of 10 mg/kg it was 36 - 38 %. After nortriptyline the inhibition of noradrenaline uptake was 29, 46, 51 and 71 % (1, 3, 10 and 30 mg/kg, resp.) The maximal inhibition of 5-HT uptake was much less, 28 % at the dose of 30 mg/kg.

The correlation coefficients between the chemically assayable amitriptyline or nortriptyline and the uptake inhibition were rather modest. A significant linear correlation was achieved only for noradrenaline uptake (amitriptyline: $r = 0.476$, $p < 0.05$; nortriptyline: $r = 0.423$, $p < 0.05$). The bioassay indicated 10 to 100-fold higher concentrations in the case of amitriptyline and 2 to 10-fold higher concentrations in the case of nortriptyline as compared to those detected with GLC-assay.

These results suggest that biological activity (uptake inhibition) is higher after the administration of amitriptyline or nortriptyline in vivo than what could be expected on the basis of the concentrations of parent compounds in plasma. The most obvious explanation which deserves more studies is the presence of remarkable and variable amounts of active metabolites in plasma.

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BILIARY EXCRETION AND ENTEROHEPATIC RECYCLING OF GLUTATHIONE CONJUGATES

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A number of drugs are known to undergo glutathione conjugation and subsequent excretion in bile (Chasseaud, 1979). The fate of these conjugates in the gastro-intestinal tract is poorly understood, but it appears that they can undergo reabsorption and metabolism, before being excreted in urine as mercapturic acids and other metabolites (Larsen & Bakke, 1981). We have investigated the fate in the GI tract of three glutathione-conjugated xenobiotics.

Bile duct-cannulated male Wistar rats were dosed i.v. with bromsulphthalein (BSP, 12 μmol/kg), [^{14}C]-1-chloro-2,4-dinitrobenzene (CDNB, 5 μmol/kg) or [^{14}C]-naphthalene (30 μmol/kg). The biliary metabolites were analysed by t.l.c. The extent of biliary excretion of these compounds and the proportion of biliary metabolites as glutathione conjugates are shown in Table 1(a).

Table 1 Excretion of metabolites of BSP, CDNB and naphthalene (Figures are Av. ± S.D.(n). N.D. = not detected)

a) Intravenous administration of parent compound

Compound	Time (h)	% Dose excreted in Bile	% Dose excreted in Urine	% Biliary metabolites as glutathione conj.
BSP	4	81.0± 5.8(3)	N.D. (3)	52.7±9.6(3)
CDNB	24	35.5± 5.3(3)	50.6±10.0(3)	40.0±2.6(3)
Naphthalene	6	63.9±11.6(7)	13.5±10.5(5)	76.0±2.6(3)

b) Intraduodenal infusion of glutathione conjugate

Glutathione conj. of	Time (h)	% Dose excreted in Bile	% Dose excreted in Urine	% Urinary metabolites as mercapturic acid
BSP	24	N.D. (3)	N.D. (3)	-
CDNB	24	28.5±6.3(3)	35.5±11.1(3)	59.8±5.2(3)
naphthalene	24	37.7±2.6(3)	44.5± 2.5(3)	81.6±4.0(3)

Glutathione conjugates of BSP, [^{14}C]-CDNB and [^{14}C]-naphthalene were infused intraduodenally into a further group of bile duct-cannulated rats. The re-excretion of the infused doses of these glutathione conjugates is shown in Table 1(b). In the case of BSP, no metabolites were detected in the bile or urine indicating the absence of enterohepatic circulation which may be due to the highly polar nature of BSP. The glutathione conjugates of CDNB and naphthalene, however, underwent metabolism during enterohepatic recycling and appeared in the bile and urine predominately as the corresponding mercapturic acids. Enterohepatic recycling of glutathione conjugates is thus important in the overall disposition and excretion of drugs as mercapturic acids in urine.

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ISOLATION OF PURIFIED ASPIRINASE FROM GUINEA-PIG LIVER MICROSOMES AND IDENTIFICATION OF A CYTOPLASMIC ASPIRINASE

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The hydrolysis of aspirin as part of its metabolism has been recognized for a long time, but little has been established about the enzymology of the reaction. The liver has been shown to contribute significantly to this step *in vivo* (Harris & Riegelman, 1969) in dog, and work in this laboratory has established that the main aspirin hydrolyzing activity in guinea pig liver can be attributed to a microsomal carboxylesterase (White & Hope, 1981). The enzyme has now been isolated from guinea-pig liver microsomes prepared by high-speed centrifugation of the post-mitochondrial supernatant using a saponin solubilizate further purified by fractionation with $(NH_4)_2SO_4$, gel filtration on Sephadex G-100, and ion-exchange chromatography on DEAE Sephadex A-50 and CM-Sepharose CL-6B. The first ion-exchange step resolved a minor peak of aspirin hydrolyzing activity, with the main peak accounting for 97% of the total activity.

The preparation was homogeneous as judged by electrophoresis on polyacrylamide gels, staining with Coomassie Blue and thioaspirin. The molecular weight determined by gel filtration is about 53,000 and an estimate of subunit molecular weight by SDS-PAGE indicates that the enzyme is monomeric.

The enzyme is not specific for the acetyl esters of salicylic acid, but shows higher activity as chain length of the acyl moiety increases.

The enzyme is very sensitive to inhibition by the carboxylesterase organophosphate inhibitor BNPP, being inhibited rapidly and irreversibly with a Ki of 10^{-7}M.

The purification confirms that aspirin and its sulphur analogue, thioaspirin are hydrolysed by the same enzyme as was earlier assumed (White & Hope, 1981).

Inoue *et al.* (1980) described an enzyme isolated from human liver which hydrolysed aspirin and some of its homologues. In contrast to the aspirinase from guinea-pig microsomes the human enzyme belonged to the high molecular weight carboxylesterases (M.W. 180,000), and esters of β naphthol were hydrolysed at least one hundred times faster than aspirin.

The cytosol fraction recovered from guinea-pig liver homogenates contained a small amount of aspirin hydrolysing activity. Electrophoresis of the fraction followed by staining with thioaspirin showed that this activity is due to a cytoplasmic enzyme and not to activity derived from the microsomes. The cytoplasmic enzyme accounted for 13.3 ± 3.9 ($n = 5$) per cent of the total activity toward aspirin and 11.9 ± 3.4 ($n = 5$) per cent toward thioaspirin.

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STUDIES ON PROTEIN BINDING OF SULFINPYRAZONE AND TWO OF ITS METABOLITES IN HUMAN SERUM AND HUMAN SERUM ALBUMIN

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During the last few years much attention has been drawn to the possible profylactic effect of sulfinpyrazone on thromboembolic disease '(Anturane Reinfarction Trial Research Group, 1980; Anturan Reinfarction Italian Study Group, 1982)'. The compound is extensively metabolized into a sulfide and a sulfone metabolite '(Dieterle et al, 1980; Jakobsen & Pedersen, 1981)', which appear to be more potent inhibitors of platelet aggregation than the parent compound '(Pedersen & Jakobsen, 1981)'.

In human serum the percentual binding of sulfinpyrazone, the sulfide and the sulfone metabolite were 99.2%, 99.8% and 98.3%, respectively. Rosenthal plots representing the binding of sulfinpyrazone and the two metabolites in solutions of human serum albumin (HSA) and human serum yielded the following values for association constants and number of binding sites.

	Human Serum Albumin				Human Serum			
	n_1	$k_1 \times 10^{-5}$ 1/mol	n_2	$k_2 \times 10^{-5}$ 1/mol	n_1	$k_1 \times 10^{-5}$ 1/mol	n_2	$k_2 \times 10^{-5}$ 1/mol
Sulfinpyrazone	0.92	2.5	4.0	0.14	0.74	3.1	4.3	0.14
Sulfide	2.3	18.3	4.5	0.11	2.1	7.6	7.2	0.04
Sulfone	0.25	5.3	4.1	0.14	0.23	3.4	4.1	0.20

The sulfide metabolite displaced the parent compound from its binding sites in solution of HSA ($3.3 \cdot 10^{-5}$M). At a concentration of 25 µM sulfide a decrease in k_1-value and an unaltered number of primary binding sites was found. At a concentration of 100 µM sulfide the k_1-value was further decreased and was approaching the k_2-value. However, no displacing effect of the sulfide metabolite on the protein bound fraction of sulfinpyrazone was found, when both compounds were added to undiluted serum in a concentration of 20 µg/ml.

Introduction of an acetyl group in human serum albumin by reaction with acetylsalicylic acid (ASA) changed the ability of HSA to bind sulfinpyrazone. The k_1-value approached the value of k_2. In vitro incubation of human serum with ASA (200 µg/ml) resulted in complete deacetylation of the ASA but no change in the ability of serum to bind sulfinpyrazone other than that caused by salicylic acid was detected.

Addition of phenylbutazone, tolbutamide or salicylic acid in concentrations above 50 µg/ml caused significant ($p < 0.05$) displacement of sulfinpyrazone (20 µg/ml).

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GASTRIN COMPONENTS IN DOG DUODENUM AFTER ANTRECTOMY

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The duodenum of dog contains only low amounts of gastrin, corresponding to approximately 1 % of the antral gastrin content. Using radioimmunoassay we have previously demonstrated that there is an increase in the gastrin concentration of the proximal part of the canine duodenum after antrectomy (Brodin & Nilsson, 1982). To reveal if there is any change in the relative amounts of different gastrin components after antrectomy gel chromatography of duodenal tissue extracts from control dogs and antrectomized dogs was performed.

In unoperated dogs (n=4), the major part (70 %) of the gastrin-like immunoreactivity, determined with a COOH-terminal directed antiserum, was eluted in a peak appearing at the same site or slightly after ^{125}I. This material most likely represents small COOH-terminal fragments of gastrin or cholecystokinin, which have been reported to elute in this region (Rehfeld & Kruse-Larsen, 1978). In all extracts from control dogs a small peak appeared in the region of gastrin-34 while gastrin-17-like immunoreactivity was seen only in two of these. In antrectomized dogs with gastroduodenostomy (n=4) or gastrojejunostomy (n=4) gastrin-17 was found to dominate while the amount of immunoreactivity found in the gastrin-34 region was only at most one tenth of that of gastrin-17. Approximately 15 % of the total gastrin-like immunoreactivity was eluted late, corresponding to the main peak in the extracts from control dogs.

The present results show that the same gastrin components are found in the canine duodenum before and after antrectomy. The increase in duodenal gastrin seen after this kind of operation is mainly due to gastrin-17.

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EFFECT OF DEGLYCYRRHIZINATED LIQUORICE ON CANINE GASTRIC MUCOSAL BLOOD FLOW AND ION PERMEABILITY

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An adequate gastric mucosal blood flow(GMBF) is one of several factors associated with protection of the gastric mucosa from injury. Deglycyrrhizinated liquorice (DGL), an extract of liquorice heals peptic ulcers in man (Morgan et al 1978), although its mechanism of action is poorly understood. We have previously reported that DGL (100mg), after topical application to the canine Heidenhain pouch causes an increase in GMBF without concurrent changes in the movement of H^+ and Na^+ ions (Johnston & McIsaac 1981). The present studies were undertaken to explore further the effect of two doses of DGL on GMBF and ion permeability.

Four female dogs with vagally denervated gastric pouches were used. Isotonic hydrochloric acid (100 mM HCl , 50 mM NaCl and 2 μCi 36-Cl) was instilled into the pouch using a continuous loop perfusion method and recovered every thirty minutes. H^+ concentration in the recovered solution was measured by titration to pH 7.0 with 0.1 N NaOH (Radiometer, Copenhagen), Na^+ by flame photometry (Corning 450) and $36\text{-}Cl^-$ by liquid scintillation counting (Packard Tricarb). Ion movements were calculated as loss or gain compared to the original solution in $\mu mol\ min^{-1}$. GMBF was estimated using the neutral red clearance technique (Knight & McIsaac 1977) and expressed in $ml\ min^{-1}$. The study consisted of seven thirty minute periods. DGL (100 or 1000 mg) was suspended in the perfusing fluid and administered during the fourth period, and collections were continued for a further three periods. The two doses were studied twice in each animal. The means of the second and third periods represent pre-drug and means of the fifth and sixth periods post-drug values and were compared using the Wilcoxon's Signed Ranks test for matched pairs (Siegel 1956). SEMs are given for comparative purposes.

The 100 mg dose of DGL had no effect on the movement of ions but was found to increase GMBF from 3.0 ± 0.8 to 5.7 ± 1.9 $ml\ min^{-1}$ ($P < 0.05$).The higher dose of DGL increased GMBF from 3.3 ± 0.5 to 8.8 ± 1.6 $ml\ min^{-1}$ ($n=8$, $P < 0.02$), with a significant increase in the loss of both H^+ and $36\text{-}Cl^-$ (6.4 ± 1.2 to 11.6 ± 1.7 $P < 0.05$, 11.0 ± 3.7 to 23.0 ± 6.6, $P < 0.05$ respectively). Na^+ gain decreased in the fourth period only (5.2 ± 1.9 to 2.8 ± 1.2, $P < 0.05$).

We can conlude that part of the increase in GMBF seen on topical application of DGL may be related to changes in the mucosal permeability allowing increased amounts of H^+ ions to enter the mucosa. The increased loss of $36\text{-}Cl^-$ and the transient decrease in Na^+ gain may be due to alterations in membrane permeability.

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INHIBITORY EFFECT OF CALCIUM ANTAGONISTS ON BASOPHIL HISTAMINE RELEASE: IN VIVO AND IN VITRO STUDIES

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Calcium antagonists such as verapamil, nifedipine, and nimodipine selectively inhibit calcium ion influx across the cell membrane. Calcium ions are essential for secretion of allergic-inflammatory mediators and for contraction of bronchial smooth muscle. The drugs might therefore be of value in asthma and other atopic diseases, and some preliminary reports indicate an improvement in exercise-induced asthma and a protective effect against histamine-induced bronchoconstriction (Patel, 1981a, b, c; Williams et al. 1981).

In the present study the influence of calcium antagonists on histamine release was investigated. The basophil histamine release technique was used (Stahl Skov & Norn, 1977). Blood leukocytes with approx. 2% basophils isolated by the Ficoll-Hypaque gradient method were incubated at 37°C for 40 min with specific antigen (cladosporium herbarum), anti-IgE or calcium ionophore A23187 in the presence or absence of calcium antagonists. With all stimulators a significant inhibition of 15 to 40% was obtained by nifedipine and nimodipine in concentrations as low as 0.3×10^{-5}M, while higher concentrations (10^{-4}M) were required for verapamil. The inhibition of histamine release was increased by higher drug concentration, and a nearly complete inhibition was often obtained by 3×10^{-5} M of nifedipine and nimodipine and by 10^{-3} M of verapamil.

In the in vivo studies 50 mg of nifedipine was given sublingually and thereafter histamine release was induced in vitro on the Ficoll-Hypaque isolated cells by anti-IgE or A23187. A significant decrease in the release of histamine (25-40%) was obtained 2 to 6 hours after drug administration.

The results indicate that calcium antagonists are able to inhibit the cellular secretion of histamine. Inhibition was obtained with nifedipine, nimodipine, and verapamil by in vitro experiments and also after peroral administration of nifedipine. The action of calcium antagonists might contribute to obtaining a beneficial effect in atopic diseases.

Nifedipine and nimodipine were generously supplied by Bayer AG, Germany and verapamil by Knoll AG, Germany.

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ADENOSINE BOTH POTENTIATES AND INHIBITS HISTAMINE RELEASE FROM HUMAN BASOPHIL LEUCOCYTES

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Adenosine has been reported to inhibit immunological mediator secretion from human basophil leucocytes (Marone et al, 1979) but to potentiate secretion from rat peritoneal and guinea-pig lung mast cells (Holgate et al, 1980; Welton & Simko, 1980). We have demonstrated that adenosine may either inhibit or potentiate mediator release from human basophils depending on the temporal relationship between adenosine addition and immunological stimulation and have examined the receptors responsible for mediating these effects.

Human leucocytes, separated from fresh venous blood as described by Lichtenstein & Osler (1964), were resuspended in Tyrode's solution containing 0.03% human serum albumin and challenged with a 1/1000 dilution of goat anti-human IgE. After 45 minutes incubation, histamine was measured spectrofluorimetrically and net percentage release calculated. Each result is a mean of at least 4 experiments.

Preincubation of leucocytes with adenosine (1×10^{-5}M) for 15 minutes prior to anti-IgE challenge reduced histamine release by 31.5 $\pm$ 9.8%. Shortening the preincubation time reduced the inhibitory activity of adenosine while addition simultaneously with anti-IgE produced no significant effect. Addition of adenosine after immunological stimulation enhanced mediator release, a maximum potentiation of 19.2 $\pm$ 7.3% being observed when adenosine was added 15 minutes after challenge. In subsequent experiments inhibition and potentiation of mediator release was assessed following drug addition 15 minutes before and after challenge respectively. The concentration-response characteristics of inhibition and potentiation were different; inhibition increased linearly with concentration whereas the potentiation curve was bell-shaped reaching a maximum at 1×10^{-6}M adenosine. That both effects are mediated through the interaction of adenosine with external membrane R-receptors rather than intracellular P-receptors (Londos & Wolff, 1977) is evidenced by: (1) the adenosine uptake blocker,dipyridamole (1×10^{-6}), did not modify either effect of adenosine; (2) theophylline (1×10^{-5}M), an R-receptor antagonist, blocked both effects; (3) N^6-(phenylisopropyl)-adenosine, an adenosine analogue which interacts with extracellular receptors only, mimics both inhibitory and potentiatory actions of adenosine; (4) the P-receptor antagonist, 2',5'-dideoxy-adenosine was inhibitory at all times of addition.

These results suggest that both inhibition and potentiation of basophil histamine release are mediated by interaction of adenosine with cell-surface R-receptors. The direction of the response depends the time of this interaction with respect to immunological stimulation.

P.J.H. is a S.E.R.C. CASE student.

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RELEASE OF PLASMINOGEN ACTIVATOR IN CONSCIOUS DOGS INJECTED WITH DDAVP

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There is convincing evidence that plasminogen activator (PA) is localized and also synthetized in the vascular endothelial cells '(e.g. Todd, 1959)' from which it may be released to the blood stream by certain vasoactive drugs such as adrenalin, nicotinic acid and vasopressin '(Nilsson & Pandolfi, 1970; Mannucci et al, 1975)'. In recent years it has been shown that i.v. administration of the synthetic analogue 1-deamino-8-D-arginine vasopressin (DDAVP) causes a marked increase in plasma concentrations of PA '(Mannucci et al, 1975)'. The present study was made to elucidate the mechanism of release of PA using DDAVP as a stimulus.

Beagle dogs with exteriorized carotid loops were accustomed to the laboratory environment and to percutaneous catheterization of arteries and veins. The dogs were standing on a table loosely supported by a canvas sling. DDAVP (25 µg in 4 ml of 0.9 % NaCl) was slowly injected over a period of 4 min and blood samples were obtained from peripheral veins before and at regular intervals after the injection.

I.v. injection of DDAVP in the jugular or femoral vein caused little or no increase in plasma concentrations of PA. However, i.a. injection of the same amount of DDAVP into the common carotid artery produced a marked increase (500-800 %) in fibrinolytic activity of venous blood with no measurable differences between jugular and femoral venous blood. Maximal concentrations of PA were observed 1 min after the injection and at 60 min the activity had returned to basal levels. Repetition of the i.a. injection of DDAVP after 60 or 120 min was without effect on the fibrinolytic activity of the blood, however, after an interval of 6 days the fibrinolytic response to i.a. injection of DDAVP was fully restored. When DDAVP was given in the femoral artery rather than in the carotid artery no changes in plasma PA could be observed.

The present data suggest that the increase in PA in plasma seen after DDAVP could be due to the release from the carotid area of supply of an agent which stimulates the endothelial cells to secrete PA. The releasable amounts of this agent appear limited and its rate of synthesis rather slow.

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AN IMPROVED MODEL FOR TESTING ANTI-INFLAMMATORY ACTIVITY OF DRUGS ON RAT EAR OEDEMA

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The "Tonelli rat ear edema test" has been a standard model for screening topical anti-inflammatory activity of drugs. Disadvantages with this model are that it is based on the potent tumour promotor croton oil and that the edema is measured in anaesthetized animals. We have developed a model based on the edema-inducing ethylphenylpropiolate (EPP), which has only low promoting activity and on an improved technique for recording the edema in conscious animals. 2 mg EPP topically on the ear induces an edema which is maximal (edema thickness 320 $\mu m \pm 49$ S.D.) after 2 h. A vascular edema is seen in the dermis and subcutis. Between 24-72 h after application there is hyperplasia of epidermis. The underlying inflammatory mediators are not known, but the edema is slightly inhibited by high systemic doses of brompheniramine (H_1-antagonist), cimetidine (H_2-antagonist), methysergide (serotonine-antagonist) but not by indomethacine (cyclooxygenase inhibitor), FPL 55712 (SRS-A-antagonist) or cytarabinum (WBC-depleting).

The edema as well as the epidermal hyperplasia is effectively inhibited by topical pretreatment of the ear with potent glucocortocoids (GCS) $\geq$ 100 ng/ear. The minimum pretreatment time for inhibition by GCS is 3-4 h, the optimum time 16 h and maximum time > 24 but < 48 h. Other types of steroid hormones had no topical anti-edema effect. The inhibition by topical GCS can be counteracted by giving high systemic doses of an anti-GCS (cortexolone).

The edema was slightly inhibited by systemic application of the β_2-stimulant terbutaline and markedly by the xanthine D-4026 and by chlorpromazine.

RECEPTOR-MEDIATED TOXICITY ON HUMAN LEUKEMIC CELLS BY LOW-DENSITY LIPOPROTEIN INCORPORATED N-TRIFLUOROACETYLADRIAMYCIN-14-VALERATE

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The major problem in cancer chemotherapy is the lacking selectivity of the drugs used, leading to severe side effects on critical normal cells. A possible way to reduce these effects would be to link the drugs to a specific carrier with a high affinity for the malignant cells. Human cells have a receptor-mediated uptake and degradation of plasma low density lipoprotein (LDL) to utilize exogenous cholesterol. We have compared the cellular uptake and degradation of ^{125}I-LDL by freshly isolated white blood cells from peripheral blood and bone marrow of leukemic patients and healthy subjects. We have found that leukemic cells from most patients with acute myelogenous leukemia (AML) have a much higher LDL receptor activity than granulocytes, mononuclear cells and non-separated bone marrow cells from healthy individuals. The enhanced LDL-receptor activity does not seem to be a common feature for malignant cells since leukemic cells from patients with acute lymphoblastic leukemia have very low activities.

Using a technique for reconstituting LDL with exogenous lipophilic substances (Krieger et al, 1979), we have incorporated the very lipophilic anthracycline derivative N-trifluoroacetyladriamycin-14-valerate (AD 32) (Israel et al, 1975) into LDL-particles. The complex, containing about 700 molecules of AD 32 per LDL-particle, moved as native LDL on agarose electrophoresis. We have studied the intracellular accumulation of AD 32 in leukemic cells from AML-patients after in vitro incubation with AD 32-LDL. When the incubation was performed in the presence of a 20-fold excess of native LDL, there was a marked reduction of the intracellular accumulation of AD 32 indicating receptor-mediated uptake of the drug. Furthermore, when the intracellular accumulation of AD 32 was plotted as a function of the concentration of AD 32-LDL in the medium, a saturable drug uptake curve was obtained similar to the curve for the intracellular accumulation of ^{125}I-LDL. Comparing the cellular uptake of free and LDL-incorporated AD 32 we found a 4-fold higher drug accumulation with AD 32-LDL complex in leukemic cells from an AML-patient with very high LDL receptor activity.

Incubation of leukemic cells with the AD 32-LDL complex in vitro inhibited the colony forming ability of the cells in semi-solid agar. This effect could be counteracted by the addition of native LDL in excess. On the other hand, native LDL did not counteract the toxic effects of free AD 32. When peripheral mononuclear cells from a healthy individual was preincubated in lipoprotein-deficient serum, the LDL-receptor activity increased and the inhibition of the colony forming ability of AD 32-LDL was more pronounced than for cells not preincubated.

Our results suggest that the LDL receptor pathway may be used to target cancer chemotherapeutics to leukemic cells for the future treatment of AML.

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PROLONGED ACTION OF DEPOLARIZING DRUGS IN GUINEA-PIG MUSCLE

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Depolarizing drugs in guinea-pig diaphragm produce a biphasic response, with a sequence comprising initial neuro-muscular block, recovery, and secondary block (Jenden 1955). Guinea-pig muscle has proved suitable for prolonged recording with micro-electrodes (2h or longer at 37°C) at the end-plate region.

Initial block with carbachol (80μM) or decamethonium (10μM) was associated with depolarization at the end-plate of 28mV (median of 12) which was followed by spontaneous repolarization in the presence of the drug such that the membrane potential returned to approximately its former level and remained steady at a time when the secondary block was occurring. The repolarization process required the presence of external potassium, as in rat muscle (Creese & Mitchell, 1981). The input resistance in the presence of decamethonium, measured by the use of two electrodes remained low despite the recovery of membrane potential.

When recovery of membrane potential had occurred in the presence of carbachol, an action potential could be recorded following nerve stimulation; at a later stage only end-plate potentials could be recorded and these eventually declined to a negligible amplitude. In parallel experiments with iontophoretic application, carbachol in the external solution produced a progressive decline in the amplitude of the iontophoretic response until a diminished though steady effect on the end-plate was produced. The methods have been used in the assessment of pre-synaptic failure and post-synaptic desensitization following long - continued application of depolarizing drugs (Hubbard, Llanas & Quastel, 1969).

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INDUCTION OF PINOCYTOSIS BY AMINOGLYCOSIDE ANTIBIOTICS

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Aminoglycoside antibiotics (AGA) accumulate by pinocytosis and damage proximal tubular cells in the kidney. The nephrotoxic potential appears to be related to the number of free aminogroups of the antibiotics (cf. Kaloyanides & Pastoriza-Munoz, 1980). Polycationic compounds may induce intensive pinocytosis and the enhanced uptake of plasma membrane that follows may be harmful to the cell. We therefore tested whether reported differences in nephrotoxicity correlate with the extent to which the drugs induce or stimulate pinocytosis in Amoeba proteus, a model cell for the study of pinocytotic processes. Amoebae were exposed to Neomycin (Neo), Gentamicin (Gent), Netilmicin (Net), Tobramycin (Tob), Kanamycin (Kan) and Streptomycin (Strep) at 1-200 µg/ml. Pinocytosis was measured in the phase-contrast microscope by counting the number of pinocytotic channels (Josefsson, 1975). The order of inducing potency of the drugs dissolved in a fresh water (Chalkley) culture medium at pH 7 was Neo > Gent > Net ≥ Tob > Kan > Strep (Fig. 1A). Ca^{2+} inhibited AGA-induced pinocytosis and counteracted the depolarisation of the amoeba membrane caused by these drugs. Pinocytosis induced by Net and Gent, in that order, was inhibited by the presence of 100 mM Na^+ and conversely, Na^+-induced pinocytosis was inhibited by Net. Consequently, pinocytosis in Ringer's solution at pH 7 (Fig. 1B) was stimulated by AGA in the following order: Neo > Gent > Tob > Kan. Net decreased the pinocytotic activity elicited by Ringer's solution (Fig. 1B, shaded line). The inhibitory effect of Net was reversed by 4-aminopyridine and a Ca^{2+}-ionophore, A23187.

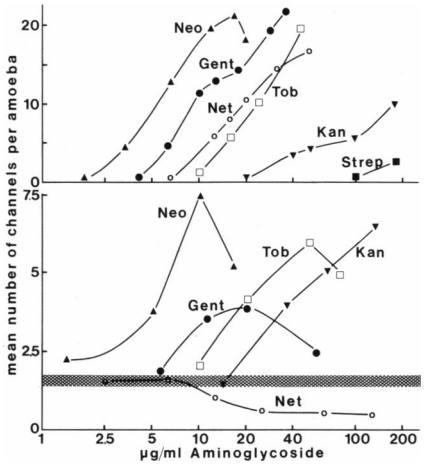

Figure 1 AGA-induced pinocytosis in (A) Chalkley's and (B) Ringer's solution.

Heparin (50 µg/ml) and carbenicillin (1 mg/ml) which are known to inactivate AGA (Regamey et al 1972, Farchione, 1981) and to counteract the nephrotoxic effect of these drugs inhibited AGA-induced pinocytosis significantly. Cephalothin (80 µg/ml) which has been claimed to increase the nephrotoxicity of AGA in man (Appel & Neu, 1977), was a weak potentiator of AGA-induced pinocytosis in the amoeba.

It is concluded that the most nephrotoxic aminoglycosides, Neo and Gent, are potent inducers of pinocytosis even in the presence of Na^+. The relative safety of Net may be related to inhibition of pinocytosis in the presence of Na^+, a possible inducer of pinocytosis in the cells of the proximal tubule.

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NEW GLUCOCORTICOIDS WITH IMPROVED RATIO BETWEEN TOPICAL AND SYSTEMIC ACTIVITIES

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Local treatment with glucocorticosteroids (GCS) is an important therapeutic principle for relieving allergic and inflammatory disorders on respiratory mucous membranes and skin. A localized effect is desired, but sometimes general GCS actions may occur owing to extensive systemic absorption. The systemic potency of a GCS has been considered to be rather closely correlated to its topical anti-inflammatory potency.

The currently used potent topical GCS are either 16α,17α-acetals of halogenated 16α,17α-dihydroxycorticosteroids (e.g. triamcinolone acetonide) or 17α-esters of halogenated 16-methyl-17α-hydroxycorticosteroids (e.g. beclomethasone 17α,21-dipropionate). Both types originate from the corresponding compounds without 16α,17α-acetal or 17α-ester substitution, which compounds basically were developed for systemic therapy.

For the 16α,17α-acetal type of GCS structure-activity investigations were performed to study the structural requirements for the topical anti-inflammatory and the systemic potency, respectively. After topical application to rats and mice, the former potency was determined as inhibition of ear edema formation and the latter as involution of the thymus. With a new type of asymmetric 16α,17α-acetal substitution it was possible to enhance the topical anti-inflammatory potency without a rise of the systemic potency. Introduction of fluoro atoms in the 9α- or especially in the 6α- or 6α,9α-positions on the other hand raised the systemic potency much more than the topical anti-inflammatory potency. The best topical/systemic ratio was obtained with the compound budesonide (16α,17α-butylidenedioxy-11β,21-dihydroxypregna-1,4-diene-3,20-dione) which had an ~10 times better ratio than triamcinolone acetonide or beclomethasone 17α,21-dipropionate.

The improved ratio between the topical anti-inflammatory and the systemic potency reached with budesonide, probably depends on a high biotransformation rate of this substance in the liver. This might at least partly depend on budesonide's lack of halogen substituents. To study the importance of liver biotransformation the systemic potency of budesonide was determined after administration by 5 different routes to rats with either normal or reduced (by SKF-525A treatment) liver biotransformation capacity. In the latter animals the systemic potency of budesonide rose 2-10 times, depending on the mode of administration. The results support the view that inactivation by biotransformation in the liver is an important reason for the relatively low systemic potency of budesonide, also when this substance is applied in the lung or on normal or damaged skin.

INFLUENCE OF DOPAMINERGIC DRUGS ON STEROID HORMONE SECRETION

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Many drugs known to modify the rate of acid secretion or the motility of the stomach have recently been shown to influence the secretion of gastrointestinal hormones. We have described changes in gastrin, insulin (Uvnäs-Wallensten & Goiny 1981), VIP (Uvnäs-Moberg et al. 1982), and somatostatin (to be published) levels in the peripheral blood of conscious dogs following administration of dopaminomimetics. Insulin induced hypoglycemia is also known to influence the release rate of several gastrointestinal hormones. Whether steroid hormones are also regulated by dopaminergic systems is still a matter of debate (see Weiner & Ganong 1978). We thus investigated whether the levels of cortisol and other adrenocorticoids are influenced by dopamine agonists or antagonists as well as by insulin induced hypoglycemia.

Indwelling catheters were inserted into superficial leg veins of conscious dogs before the experiments were started to allow blood sampling and administration of drugs. Blood samples were collected in ice chilled tubes containing heparin. Steroid hormones were assayed radioimmunologically either with or without a previous chromatographic purification to assess the identity of the hormones detected. Apomorphine (0.05 mg/kg) produces a 60 min long increase of cortisol level. Bromocriptine (0.1-0.2 mg/kg) has a more prolonged effect, cortisol levels still being maximal ($\sim$70 ng/kg) 60 min after injection of the drug. Atropine (0.1 mg/kg), methysergid (0.1 mg/kg), nalorphine (0.1 mg/kg) as well as adrenergic antagonists have no apparent effect on these cortisol responses. The dopamine antagonists halopemid (0.1 mg/kg), domperidone (0.1 mg/kg) and sulpiride (0.5 mg/kg) known to affect mainly, in such doses, peripheral dopaminergic receptors totally block the cortisol response to apomorphine (0.05 mg/kg) and to bromocriptine (0.2 mg/kg). Dopamine antagonists known to block dopamine receptors within the CNS, such as haloperidol (0.1 mg/kg) and flupenthixol (0.1 mg/kg) cause a longlasting increase of cortisol levels by themselves in addition to blocking the apomorphine induced increases of cortisol levels.

During insulin hypoglycemia a gradual rise of cortisol level occurs. Insulin (0.6 IU/kg) administered to non starved dogs produces a rise of cortisol levels from 20 to 50 ng/ml which lasts for $\sim$60 min. Adrenergic antagonists, serotonine antagonists (methysergid and cyproheptadine 0.1 mg/kg), atropine, pimozide (0.1 mg/kg) domperidone and halopemide do not seem to inhibit the increase of cortisol levels induced by insulin hypoglycemia. However, the cortisol responses induced by the centrally acting dopamine antagonists flupenthixol (0.1 mg/kg) and chlorpromazine (1 mg/kg) were completely abolished by the insulin induced hypoglycemia.

Progesterone levels were increased in parallel to cortisol levels in dogs of both sexes, following administration of dopaminomimetics or following insulin induced hypoglycemia. The levels of testosterone and oestradiol were not significantly influenced.

Our results show that the secretion of cortisol and progesterone are influenced by dopaminergic drugs. The sites of action are actually unknown but both peripherally and centrally induced effects are suggested.

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THYROID HORMONE AFFECTS Na^+, K^+-ATPase FUNCTION IN DEVELOPING NERVOUS TISSUE IN VITRO

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Thyroid hormone has a wide range of effects on neuronal function in both developing and mature central nervous tissue. (see Atterwill, 1981). One important parameter under thyroid hormone control is Na^+,K^+-ATPase, a key plasma-membrane located enzyme controlling ion metabolism and electrical activity in developing brain (Abdel-Latif et al, 1967; Valcana & Timiras, 1969). An understanding of the hormonal effects at the molecular level is often difficult from *in vivo* studies because of tissue heterogeneity and the complexity of secondary reactions encountered *in vivo*. An alternative approach is the use of *in vitro* tissue culture where it is possible to obtain CNS derived cultures whose cellular composition is well characterized and to support these cells in chemically-defined media (Bottenstein & Sato, 1979). We have, therefore, studied the action of tri-iodothyronine (T_3) on the Na^+, K^+-ATPase of nerve cells grown in such media in both rat cerebellar monolayer cultures and foetal whole-brain reaggregate cultures.

Cerebellar monolayer cultures enriched in granule cells were grown in a serum-free medium based on that of Bottenstein & Sato (1979) on poly-L-lysine coated dishes following cell dissociation from trypsinized postnatal tissue. Foetal brain reaggregate cultures from mechanically dissociated tissue were grown in a similar medium to the method of Honegger & Lenoir (1980). T_3 (2 or 30nM) was added at 2 days *in vitro* (2 DIV; surface cultures) or 3 DIV (reaggregate cultures) and cells were harvested at subsequent times for assay. Na^+, K^+-ATPase activity of cell membranes was measured radiometrically using [^{32}P]-ATP, [^{3}H]ouabain binding was measured on cultured granule cells *in situ*, and intracellular Na^+ and K^+ concentrations were measured by flame photometry.

The serum-free granule cell cultures differed morphologically from cells grown in the presence of foetal calf serum with little or no aggregation of cell bodies or fasiculation of neurites. Addition of 2nM T_3 evoked a marked increase in the specific activity of Na^+, K^+-ATPase in these cells (approx. 200% above control serum-free cultures at 6 DIV). Similarily, T_3-treated (30nM) aggregate cultures showed increases in enzyme activity of 60-100% above serum free controls and to a level similar to that found in serum-supplemented cultures. Preliminary results indicated that both ion movements and [^{3}H]ouabain binding were affected in the monolayer cultures. There was a decrease in the intracellular Na^+/K^+ ratio from 2.8 (serum-free control) to 0.6 (T_3-treated cells). [^{3}H]ouabain binding to the cultured granule cells showed that T_3 treatment produced an increase in the number of enzyme molecules (Bmax) with no change in affinity for ouabain (K_D).

Thus it appears that T_3 can promote the differentiation of this membrane property of nerve cells grown in the absence of serum. Whether this change is specific for the Na/K pump is not yet known. We are also testing for this T_3 effect on cultured glial cells in order to pinpoint the precise cell type on which T_3 is acting.

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INFLUENCE OF THE HORMONAL BALANCE OF LATE PREGNANCY ON THE ACTIVE UPTAKE OF 5-HYDROXYTRYPTAMINE BY HUMAN BLOOD PLATELETS

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Hormonal factors, especially steroid hormones, have been shown to influence the active uptake of 5-hydroxytryptamine (5-HT) both in platelets and in certain parts of the CNS. Adrenalectomy and hypophysectomy decrease the uptake of 5-HT and the effect can at least to some extent be prevented by corticosterone (Vermes et al, 1976; Tukiainen, 1980). Oral contraceptives containing oestrogen and progesterone increase both the amount of 5-HT receptor sites and the uptake of 5-HT by human blood platelets (Peters et al, 1979).

In this study, the active uptake of 5-HT by blood platelets was studied in vitro in the blood samples of twelve women during the last two months of pregnancy. The control group consisted of twelve women, who had given birth on an average four days before the collection of blood samples. The uptake of 5-HT was determined according to the method described by Tuomisto et al (1979).

There was no significant difference in the uptake of 5-HT by blood platelets of pregnant women compared to that of controls. The K_m values of both groups were 0.32 µM. The V_{max} value was 38 pmol/5 min during pregnancy, and in controls 37 pmol/5 min per $2 \cdot 10^8$ platelets.

The high blood contents of oestrogen and progesterone during the late pregnancy had thus no effect on the uptake of 5-HT by human blood platelets. As the uptake of 5-HT by blood platelets of depressed patients has been shown to be lowered, it has been suggested, that changes in blood oestrogen and progesterone contents might cause affective disorders, such as post-partum depression by having an effect on the uptake of 5-HT (Peters et al, 1979). Within the scope of the test arrangement, the present results do not give any obvious support for this hypothesis.

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PROPERTIES OF PLATELET ACTIVATING FACTOR (PAF-ACETHER) WHICH SUGGEST INVOLVEMENT IN CHRONIC INFLAMMATION AND PERSISTENT ASTHMA

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PAF-acether was described initially as a product of allergen-induced basophil degranulation. However, with elucidation of its chemical structure, it has been established that, in addition to the thrombocyte, both neutrophils and macrophages can generate PAF-acether (Vargaftig et al.,1981). These observations have prompted us to determine the effects of synthetic PAF-acether on plasma protein extravasation (PPE) and on accumulation of platelets, or leucocytes, in both guinea-pig and human skin as well as in guinea-pig lung in vivo or during in situ perfusion.

PAF-acether (1-100 ng/site) induced significant acute (0-30 min) extravasation of 125I-human serum albumin in guinea-pig skin and caused dose-related (10-1000 ng/ml) extravasation of 125I-human fibrinogen in the perfused lung (0-15 min). In human volunteers, intradermal PAF-acether (1-100 ng/site) produced wheal and erythema within 15 min.

Intravenous injection of PAF-acether (10-100 ng/kg) caused accumulation of 111In-labelled platelets in the thorax of the guinea-pig. Following the highest dose of PAF-acether, histological and electron microscopic examination of the lung showed intravascular platelet aggregation associated with neutrophil accumulation and similar effects were noted in skin lesions following injection of PAF-acether (100 ng/site). Although, in the guinea-pig, the bronchoconstrictor effect of PAF-acether is platelet dependent (Vargaftig et al.,1980), the extent to which PPE responses in guinea-pig lung or skin are dependent upon platelets remains to be resolved and we have yet to exclude the possibility that PAF-acether has a direct effect on vascular endothelium.

In addition to these acute events, preliminary evidence indicates that there are late-onset effects manifest as cellular infiltration in guinea-pig skin, and as erythema and hyperalgesia in human skin. Thus, PAF-acether has properties that are appropriate to a mediator not only of acute inflammation and asthma, but also of more sustained responses involving cellular infiltration.

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HYPOALGESIA IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) IS NOT RELATED TO BLOOD PRESSURE

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Opioid peptides appear to be involved in central cardiovascular control (Bolme et al., 1978), one of the sites of action being located in the nucleus tractus solitarii in the brain stem (Petty and de Jong, 1982). Since these peptides are also involved in pain regulation, it has been suggested that cardiovascular function and pain sensitivity might be interrelated (Zamir et al., 1979; 1980).

This hypothesis has been investigated in the present study. Using a hot plate method a decreased pain sensitivity was observed in adult (12 weeks old) Spontaneously Hypertensive Rats (SHR) as compared to their age matched normotensive Wistar-Kyoto (WKY) controls as determined by the latency times to reaction (paw licking) being 15.4 ± 0.9 and 7.6 ± 0.5 sec respectively ($p < 0.01$). Systolic blood pressure - measured in conscious animals by means of a tail sphygmographic method - was 214 ± 3 mm Hg in the SHR and 135 ± 2 mm Hg in the WKY. Administration of the opiate antagonist naloxone (1 mg/kg, s.c.) reduced the hot plate response latency in SHR to the level of WKY, indicating the involvement of opioid peptides. No effect on blood pressure was observed. Up to the age of approximately 4 weeks the blood pressure of SHR was not different from that in WKY, although the increased hot plate response latency time was already present ($p < 0.01$).

The addition of hydralazine (160 mg/l) to the drinking water of SHR (4 weeks of age) partially prevented the rise in blood pressure, being 161 ± 6 mm Hg in the hydralazine treated animals and 223 ± 4 mm Hg in the non-treated group ($p < 0.01$). However, the hot plate response latencies were identical in both groups, despite the difference in blood pressure. Similar results were obtained after inhibition of angiotensin converting enzyme with captopril (500 mg/l) added to the drinking water. In the captopril treated SHR systolic blood pressure was 149 ± 4 mm Hg, while in the control animals the usual rise in blood pressure was observed, reaching a value of approximately 220 mm Hg at the age of 9 weeks. In WKY rats the effects of hydralazine and captopril treatment on blood pressure were much smaller than in SHR, and both treatments failed to change the pain sensitivity of WKY rats.

It is concluded that the decreased pain sensitivity in SHR as compared to WKY is mediated by opioid peptides and that this hypoalgesia is not directly related to the high blood pressure. Pain sensitivity appears to be dissociated from blood pressure regulation.

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INHIBITION OF BOMBESIN- AND FAT-STIMULATED RELEASE OF NEUROTENSIN-LIKE IMMUNOREACTIVITY BY SOMATOSTATIN IN THE RAT

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Neurotensin is a tridecapeptide localized mainly in specific endocrine cells in the distal small intestine. Ingestion of fat and i.v. administration of bombesin increases the plasma concentration of neurotensin-like immunoreactivity (NTLI). (Rosell & Rökaeus 1979; Rökaeus 1980; Rökaeus et al 1982). Somatostatin, on the other hand, inhibits the release of several hormones. The aim of the present study was to investigate if somatostatin may inhibit the bombesin- and fat-induced NTLI response in the rat. NTLI was determined with the N-terminally directed antibody "0-7709" (Rökaeus 1981).

Infusion of somatostatin (30-120 pmol/min) in rats (350-500 g) started 9 min prior to i.v. injection of bombesin and continued to 5 min after the bombesin injection. Compared to control (i.v. saline infusion) somatostatin significantly reduced the integrated plasma NTLI response (pM_{0-5min}). The integrated responses for somatostatin and saline treated animals were 52±19 (n=6) and 212±32 (n=5) respectively, after administration of 25 pmol/kg bombesin ($p<0.01$) and 210±28 (n=6) and 378±53 (n=4) respectively, after administration of 125 pmol/kg bombesin ($p<0.05$). Gel filtration (Sephadex G-25) of plasma drawn at 1, 2 and 5 min (n=2 for each time) after i.v. injection of bombesin (125 pmol/kg) revealed three peaks. The peaks eluted in the order void volume (constant in size at the different times), at the position of NT (dominating peak at 1 min) and at the position of (Gln^4)-NT(1-8) (dominating peak at 2 and 5 min).

Rats (n=8) received an intraduodenal infusion of oleic acid (0.5 ml) between 0 and +10 min to stimulate NTLI release. Infusion of somatostatin (30 pmol/min. i.v., n=2) between +20 and +80 min significantly lowered the plasma concentration of NTLI at +80 min as compared with control animals (i.v. saline infusion, n=6).

In summary, i.v. injection of bombesin may release immunoreactive material which is chromatographically indistinguishable from NT. Somatostatin effectively reduces the bombesin-stimulated NTLI release and seems also to reduce the fat-stimulated NTLI release.

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EFFECTS OF SOMATOSTATIN ON THE GASTROINTESTINAL MICROCIRCULATION OF THE RAT

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Somatostatin has been shown to be therapeutically useful in the treatment of severe gastrointestinal haemorrhage in man (Gyr et al, 1981). The mechanism of action is not fully understood, but it has been shown to reduce splanchnic blood flow in both the dog and human (Doertenbach et al, 1981, Gyr et al,1981). The study reported here was carried out to assess the effects of somatostatin on blood flow through the gastrointestinal microcirculation of the pentobarbitone anaesthetised rat.

Normally fed male Sprague-Dawley derived rats (200-250g) were used throughout. Microspheres labelled with 46-Sc (15±1.3μm in dia. in dextran 40) were injected into the left ventricle of the heart according to the method of Lucas & Foy (1977). Thirty seconds later the rat was killed and various sections of the gastrointestinal tract and other organs excised, washed and weighed. Tissue radioactivity was determined using a Packard 2040 Gamma Counter. The peptide was adminstered in a solution containing 0.5% bovine serum albumin, 0.9% NaCl, 5mM HCl and 20μl methanol/mg of peptide. Peptide or vehicle alone as control was injected as a bolus (0.6 ml) into the femoral vein 30s before the microspheres. Results were expressed as percentage cardiac output per 100g of tissue (%CO) and compared to control.

Somatostatin 5μg/kg and 10μg/kg reduced the %CO to the stomach to 82% ($P<0.1$,n=6) and 84% ($P<0.05$,n=6) of the control value respectively. The only other organ affected by these doses was the kidney (increased to 116% of the control value, $P<0.05$,n=6). At higher doses of somatostatin there was no reduction in gastric blood flow but the flow through certain other tissues was increased. For example at 50μg/kg the %CO to the kidney oesophagus and duodenum was 130% ($P<0.01$,n=6) 332% ($P<0.01$,n=6) and 144% ($P<0.01$,n=6) of the control value respectively.

It would appear that somatostatin has two effects on blood flow through the gastrointestinal microcirculation of the rat. The first, a decrease in gastric blood flow, is consistent with previous reports in other species (Konturek et al, 1978). Secondly, at higher doses, there is a marked increase in flow to the duodenum and oesophagus, not previously reported. Work is continuing to further elucidate the mechanism of action of somatostatin on regional microcirculation in the rat.

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VAGAL SUBSTANCE P NERVES INVOLVED IN CONTROL OF VASCULAR PERMEABILITY AND SMOOTH MUSCLE TONE IN TRACHEA AND BRONCHI

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The trachea and lower respiratory tract contain nerve endings in the smooth muscle layers as well as under and within the lining epithelium. Activation of irritant receptors by chemical agents or foreign particles leads to local and vagal protecting reflexes resulting in e.g. bronchoconstriction and vascular reactions '(see Widdicombe, 1975)'. The chemical transmitter of such sensory neurones is not known. Substance P (SP)-immunoreactive (IR) nerve fibres are, however, present within the guinea-pig tracheo-bronchial wall '(Nilsson et al., 1977)', SP-IR cell bodies in the nodose ganglion and SP-IR axons in the vagus nerve '(Lundberg et al., 1978)'. In the present study it was shown by immunohistochemistry that SP-IR nerve fibres were present in the smooth muscle layer around blood vessels as well as under and within the respiratory epithelium of the trachea and bronchi in rat, guinea-pig, cat and man. SP-IR axons were found in the cervical vagus nerve, SP-IR cell bodies in the nodose ganglion and SP-IR nerve endings in the nucl. tractus solitarii of the brain stem of all four species. Unilateral vagotomy in rat and guinea-pig caused a considerable loss of SP-IR nerves in the trachea and lower respiratory tract. Neonatal capsaicin treatment of rats resulted in an almost total loss of SP-IR nerves in the tracheobronchial wall. VIP-ergic and noradrenergic nerves were, on the other hand, not reduced by this treatment. Evans blue (20 mg x kg^{-1}) was injected i.v. into rats. The distal end of the cut right cervical vagus nerve was stimulated (10 V, 10 Hz, 0.2 ms) for 5 min. Immediately after stimulation the rats were rinsed with 40 ml saline via the aorta. Trachea and the right lung excised and weighed. Evans blue was extracted by formamide and quantified spectrophotometrically. Vagal stimulation induced a massive Evans blue extravasation in the trachea (100± 11 µg x g^{-1}, n=4) and stem bronchi suggesting an increased vascular permeability. A similar extravasation (99 ± 19µg x g^{-1},n=4) was also seen in atropine (0.5 mg x kg^{-1}) pretreated animals. No extravasation of Evans blue was observed in control animals, where the vagus nerve was cut, but not stimulated. Fluorescence microscopy of sections from formalin-fixed tissues revealed that red fluorescence due to Evans blue extravasation was mainly present in the subepithelial layer of the trachea and bronchial tree. In neonatally capsaicin-treated rats, no Evans blue extravasation in the respiratory tract was seen after vagal nerve stimulation. Local irritation by cutting the tracheal wall or tracheal cannulation of the animal caused massive extravasation per se. Local injections (10 µl) with a glass micropipette into the tracheal lumen of SP (75 pmol) caused a significant Evans blue extravasation versus saline (25 ± 11, v. 6 ± 5 µg x g^{-1},n=3). Capsaicin which acutely activates unmyelinated chemosensitive afferent fibres causing e.g. SP release from central branches of sensory neurons '(Gamse et al., 1979) injected into lumen (300 pmol) also caused extravasation (61 ± 2 µg x g^{-1},n=3). SP and capsaicin were also found to cause bronchial smooth muscle contraction in vivo and in vitro. In conclusion, the present data suggest that activation of capsaicin-sensitive sensory SP neurones of vagal origin causes increased local vascular permeability and smooth muscle contraction in the respiratory tract. Such local axon reflexes involving SP release may be of importance for asthma and bronchial hyperreactivity symptoms.

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MEASUREMENT OF NEUROPEPTIDES BY HPLC WITH ELECTROCHEMICAL DETECTION

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Recent studies have indicated that many neuropeptides are electroactive, and that the electrochemical oxidation potentials of these peptides (+0.6 to +1.0V) are distinct from those of biogenic amines (+0.2 to +0.5V) and are associated with the presence of the amino acids tryptophan, tyrosine and cysteine (1). The differential hydrophobicity of neuropeptides results in their separation by reverse-phase HPLC (2) and tissue neuropeptides have been measured in HPLC fractions by radioimmunoassay (3). With the sensitivity of electrochemical detection (ECD), it should be possible to measure tissue neuropeptides directly by reverse-phase HPLC with on-line ECD (LCEC). In the present investigation an LCEC assay has been developed to study the separation and identification of neuropeptides in extracts of rat neurohypophysis.

Reverse-phase HPLC was carried out on a Hypersil (5 ODS) column (25 cm x 5 mm) using a 0.15M acetate/citrate buffer - methanol mobile phase (60:40), at pH 4.6; flow rate 1.0 ml/min. ECD was with a BAS glassy carbon electrode, with an applied voltage of +1.00V. Single neurohypophyses were sonicated in 1 ml extraction medium, followed by centrifugation at 3,000 r.p.m. for 15 mins. The supernatant was dried down and the residue taken up in 1 ml of acetate/citrate buffer, pH 4.6; 100 µl of this was injected onto the column. The following extraction media were compared: i) 0.01 M HCℓ, methanol (50/50 (v/v)); ii) 0.1 M acetic acid: methanol (50/50 (v/v)); iii) 0.1% trifluoroacetic acid (TFA), 40% Methanol; iv) 0.1% TFA, 80% methanol and v) 0.1% TFA, 80% ethanol. The latter has been adopted for routine experiments as it resulted in the optimum recovery of several neuropeptides in the tissue extracts.

Five peaks observed in the neurohypophyseal extracts have the following R_T values: 1) 2.5 min, 2) 4.5 min, 3) 9.5 min, 4) 14.0 min and 5) 27.5 min. Tentative identification of these has been made by spiking tissue extracts with neuropeptide standards (50 pmols/100 µl) and vasopressin and oxytocin have been found to co-elute with peaks 1) and 4). Met-enkephalin, and Leu-enkephalin and angiotensin II elute closely to, but are separable from, peaks 2) and 3) respectively. Peak 5) was eluted a considerable time after the first four peaks, and was observed as a broad band; the retention times of renin substrate, angiotensin I and neurotensin all fall within the time span of this peak. The vasopressin peak was not present in neurohypophyseal extracts prepared from Brattleboro rats and quantitative changes in the oxytocin and unidentified peaks were also observed.

The sensitivity of detection for the neuropeptides is in the range of 1 to 10 pmols (signal to noise 3:1), with an estimated sensitivity for vasopressin and Met-enkephalin of 1 pmol, and for angiotensin II and neurotensin about 5 pmols. An electrochemical assay for neuropeptides could provide an alternative approach for peptide measurement to radioimmunoassay methods - so avoiding the problems of antibody specificity.

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We have previously demonstrated increased amounts of arachidonic acid, and prostaglandins D_2, E_2, $F_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ in human skin at different times after ultraviolet irradiation (Camp et al, 1978; Black et al, 1980 & 1981). The increase in prostaglandins was coincident with the development of erythema. Although biologically active on human skin, the effect of longer wavelength infra-red irradiation (IR) on prostaglandin levels in human skin has not previously been investigated.

Volunteers were irradiated on the forearm using an Osram Theratherm infra-red lamp for 1 hour. Mean skin surface temperature was elevated to 38 2°C. Exudates were obtained from control and irradiated skin and quantitated for prostaglandins D_2, E_2, $F_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ as described previously (Camp et al, 1978; Black et al, 1980 & 1981).

Table 1

Effect of IR irradiation on the concentrations of PGD_2, E_2, $F_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ in human skin exudates. Values (ngm^{-1}) represent means s.e. means, n = number of samples; p values (student's "t" test) represent significance relative to controls.

	Time after Irradiation (hours)				
	Control	4.5	8	16	24
PGE_2	13 ± 1 n = 19	54 ± 6 n = 6 p < 0.001	98 ± 16 n = 3 p < 0.001	57 ± 14 n = 4 p < 0.001	34 ± 6 n = 4 p < 0.001
$PGF_{2\alpha}$	13 ± 1 n = 19	38 ± 3 n = 6 p < 0.001	43 ± 7 n = 4 p < 0.001	42 ± 7 n = 4 p < 0.001	29 ± 5 n = 4 p < 0.001
PGD_2	14 ± 4 n = 18	34 ± 5 n = 6 p < 0.001	52 ± 3 n = 4 p < 0.001	46 ± 15 n = 4 p < 0.005	33 ± 10 n = 4 N S
6-oxo-$PGF_{1\alpha}$	9 ± 1 n = 19	34 ± 5 n = 6 p < 0.001	26 ± 5 n = 4 p < 0.001	16 ± 2 n = 4 p < 0.001	21 ± 8 n = 4 N S

The results show that IR irradiation caused increased concentrations of prostaglandins in human skin, however, their role in the observed inflammatory action remains to be established.

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