bating the microsomal suspensions with antipyrine, were found to be unnecessary. Antipyrine is not oxidized by the P450 variant responsible for the oxidations of sparteine and debrisoquine[11, 18, 25].

Radioreceptor assays are generally best for ligands having a dissociation constant, or K_D, less than 5 nM[20–22], permitting the use of low concentrations of ^{3}H-ligand (below 2 nM) and minimizing non-specific binding. The major difficulty with ^{3}H-dihydroquinidine as a ligand is the K_i value, as estimated by its competitive inhibition of sparteine oxidation, of 40 nM (data not shown). This high value predicted the significant noise problem encountered in the binding of this radioligand and necessitated the use of a large number of replicates. As shown in the figure, the data still had a large standard error which prevented quantitative comparisons between the 2 types of assays. However, the Ki of quinidine estimated from its inhibition of sparteine and debrisoquine oxidations (30–60 nM) is within the concentration range over which quinidine inhibited ^{3}H-dihydroquinidine binding (10–500 nM).

The high signal to noise ratio may have been reduced had a more purified preparation of cytochrome P450 been available to us for use. However, having now obtained a prototype ligand, it is possible to proceed with the design of related ligands having higher oil/water partition coefficients, and thus lower K_D values. Ultimate identification of the binding component of the liver microsomal suspension awaits the synthesis of such a ligand.

These observations indicate that the techniques developed for the in vitro study of drug receptors will be applicable to the assessment of membrane-bound enzymes. Such assessment promises to be useful for the study of genetically-variable components of catalytically-inactive systems, as shown here for a member of the cytochrome P450 family. Furthermore, these observations suggest that the enzymatic handling of natural drug substrates (in contrast to artificial substrates) is worthy of attention.

1 Acknowledgments. We thank C. Ulpian for advice and assistance. We also thank Dr M. Robinette of Metro Organ Retrieval and Exchange and Dr T. Inaba for making human hepatic tissue available. This work was supported by grants from the Medical Research Council of Canada.

2 To whom correspondence should be addressed.
3 Eichelbaum, M., Clin. Pharmacokinet. *7* (1982) 1.
4 Mellström, B., Bertilsson, L., Säwe, J., Schulz, H-U., and Sjöqvist, F., Clin. Pharmac. Ther. *30* (1981) 189.
5 Lennard, M.S., Silas, J.H., Freestone, S., Ramsay, L.E., Tucker, G.T., and Woods, H.F., New Engl. J. Med. *307* (1982) 1558.
6 Alvan, G., von Bahr, C., Seideman, P., and Sjöqvist, F., Lancet *1* (1982) 333.
7 Shah, R.R., Oates, N.S., Idle, J.R., and Smith, R.L., Lancet *1* (1982) 508.
8 Shah, R.R., Oates, N.S., Idle, J.R., Smith, R.L., and Lockhart, J.D.F., Br. med. J. 284 (1982) 295.
9 Sloan, T.P., Mahgoub, A., Lancaster, R., Idle, J.R., and Smith, R.L., Br. med. J. *2* (1978) 655.
10 Otton, S.V., Inaba, T., and Kalow, W., Life Sci. *34* (1984) 73.
11 Boobis, A.R., Murray, S., Kahn, G.C., Robertz, G.M., and Davies, D.S., Molec. Pharmac. *23* (1983) 474.
12 Murray, S., Kahn, G.C., Boobis, A.R., and Davies, D.S., Int. J. Mass Spectrom. Ion Phys. *48* (1983) 89.
13 Otton, S.V., Inaba, T., and Kalow, W., Life Sci. *32* (1983) 795.
14 Nakano, M., and Inaba, T., Can. J. Physiol. Pharmac. *62* (1984) 84.
15 Inaba, T., Otton, S.V., and Kalow, W., Clin. Pharmac. Ther. *27* (1980) 547.
16 Bertilsson, L., Dengler, H.J., Eichelbaum, M., and Schulz, H.-U., Eur. J. clin. Pharmac. *17* (1980) 153.
17 Eichelbaum, M., Bertilsson, L., Säwe, J., and Zekorn, C., Clin. Pharmac. Ther. *31* (1982) 184.
18 Otton, S.V., Inaba, T., Mahon, W.A., and Kalow, W., Can. J. Physiol. Pharmac. *60* (1982) 102.
19 Inaba, T., Vinks, A., Otton, S.V., and Kalow, W., Clin. Pharmac. Ther. *33* (1983) 394.
20 Seeman, P., Chau-Wong, M., and Moyyen, S., Can. J. Physiol. Pharmac. *50* (1972) 1181.
21 Seeman, P., Chau-Wong, M., Tedesco, J., and Wong, K., Proc. natl Acad. Sci. USA *72* (1975) 4376.
22 Seeman, P., Pharmac. Rev. 32 (1980) 229.
23 Mukherjee, C., Caron, M.G., Coverstone, M., and Lefkowitz, R.J., J. biol. Chem. *250* (1975) 4869.
24 Lefkowitz, R.J., Fedn Proc. *37* (1978) 123.
25 Davies, D.S., Kahn, G.C., Murray, S., Brodie, M.J., and Boobis, A.R., Br. J. clin. Pharmac. *11* (1981) 89.

0014-4754/84/090973-02$1.50 + 0.20/0

Do prostaglandins mediate the somatostatin preventive effect on gastric lesion?

S.F. Aliño

Department of Histology, Faculty of Medicine, University of Pais Vasco, Lejona, Vizcaya (Spain), 25 October 1983

Summary. The inhibition of endogenous prostaglandin synthesis by indomethacin treatment blocks the somatostatin preventive effect on the gastric lesions induced in a stress model and has no preventive effect on an intragastric distension model.
Key words. Rat; prostaglandins; gastric lesion; intragastric distension model; stress model; indomethacin; somatostatin preventive effect.

Somatostatin is a polypeptide that acts at a gastric level in 2 important ways: inhibition of acid secretion and antiulcer activity. Recently it has been verified[1] that exogenous somatostatin stimulates synthesis and endogenous release of prostaglandin F_2 and that indomethacin (a cyclooxygenase inhibitor) blocks the somatostatin inhibitor effect on gastric acid secretion. These authors suggest that endogenous prostaglandins may be mediators in the somatostatin inhibitor effect on gastric acid secretion. To find out whether prostaglandins are also mediators in the somatostatin antiulcer effect at gastric level we examined the somatostatin activity on a stress gastric lesion model, with or without prior indomethacin treatment. Moreover we have studied this using a gastric lesion model independent of the acid inhibitory properties of the drug tested. In this model mucosal erosions were produced by intragastric distension on a continuously perfused simulated gastric juice.

Material and methods. In the stress model a male Wistar rat (200–250 g) group (n = 20) was immobilized in rigid plastic tubes and kept at 4 ± 1 °C for 3 h. 10 of these animals were treated with somatostatin (initial bolus of 2.8 μg/kg then kept at 10 μg/kg/h, i.v.) and the others treated with the same volume of saline solution. Other rats (group n = 20) were kept under the same conditions after an indomethacin treatment (5 mg/kg, i.m.) 2 h before.

In the intragastric distension model lesions were induced on anaesthetized (urethane 1.6 g/kg, i.m.) male Wistar rats

(200–250 g) by continuous intragastric perfusion (0.5 ml/min via oesophagus) of a simulated gastric juice (0.1 M HCl plus 600 mg pepsin/l) for 3 h. Intragastric pressure was applied by placing the open end of the outlet of the duodenal catheter 120 mm above the stomach level. Two groups of 10 animals each were marked out. One was treated with saline solution. At the end of the experiment, the rats were killed, the abdomen opened and the stomach removed and cut open along the greater curvature. The length (mm) of the erosions in each stomach was measured with the aid of a dissecting microscope (magnification × 10). When punctuate lesions were found, an arbitrary value of 1 mm was assigned to every 3 such lesions and the lesion index was obtained as the total length of the lesions in each stomach. Student's t-test was used to estimate the differences in the mean values of the lesion index between the groups. The research team measuring the lesions was unaware of the treatment being administered.

Results and discussion. In the stress model (table), somatostatin clearly reduced the lesion index (inhibition of 75%, $p < 0.01$). In animals previously treated with indomethacin the somatostatin failed to inhibit ($p > 0.05$) the lesions with regard to the control group. These results back up the observations made by Ligumsky et al.[1] and support the hypothesis that arachidonic acid metabolites from the cyclooxygenase pathway may be mediators of the somatostatin effect on the gastric lesions.

Effect of somatostatin on gastric lesion induced by stress and acid + gastric distension. Values are the mean ± SE of the lesion index (n = 10 for each group)

Gastric lesion model	Control	Somatostatin
Stress	8.81 ± 3.30	2.13 ± 0.85*
Stress + indomethacin	17.66 ± 3.29	12.15 ± 3.85
Gastric distension	10.14 ± 3.12	9.25 ± 4.58

* Significant difference from control group, $p < 0.01$.

In contrast, we observed (table) that somatostatin failed to reduce the gastric lesions brought about by the intragastric distension model in which exogenous prostaglandin E_2 produces a clear cytoprotective effect[2]. This failure backs up the explanation that in the stress model, somatostatin-induced protection was due to inhibition of acid secretion which is mediated by endogenous prostaglandins[1]. But our results do not exclude the possibility of the somatostatin failure being due to an inhibition of the arachidonic acid metabolism mediated by the intragastric acid medium in the rat. This has been observed by Konturek et al.[3] in the cat. In this sense also an identical behavior of somatostatin release induced by intragastric acid medium has been observed in both species[4,5].

We suggest that in the rat, the somatostatin preventive effect on the mucosal gastric lesions may be, at least in the stress model, mediated by endogenous prostaglandins. But further studies concerning the effect of intragastric acid medium on arachidonic acid metabolism at the rat gastric level are still needed to clarify the prostaglandin mediator mechanism.

1 Ligumsky, M., Goto, Y., Debas, H., and Yamada, T., Science *219* (1983) 301.
2 Marti-Bonmati, E., Aliño, S.F., Lloris, J.M., and Esplugues, J., Eur. J. Pharmac. *68* (1980) 49.
3 Konturek, S.J., Radecki, T., Brzozowski, I., Piastuchi, A., Dembinskakiec, A., Zmuda, A., and Gryglewski, R., Digestion *21* (1981) 205.
4 Aliño, S.F., Garcia, D., and Uvnäs-Moberg, K., Acta physiol. scand. *117* (1983) 491.
5 Uvnäs-Wallenstein, K., Efendic, S., and Luft, R., Acta physiol. scand. *99* (1975) 126.

0014-4754/84/090974-02$1.50 + 0.20/0

Characterization of the third component of pig complement and its utilization in a C3b receptor study

J. Zídková, V. Větvička, P. Rossmann, V. Dlabač and J. Stránsky

Institute of Microbiology, Department of Immunology, Czechoslovak Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, (Czechoslovakia), 2 November 1983

Summary. The third component of the pig complement system (C3) was isolated in hemolytically active form and characterized. The C3 component is a β-globulin with the molecular weight of 191,000 and is composed of 2 non-identical polypeptide chains of M_r 112,000 and 74,000. The isolated C3 can be used for the detection of the C3b receptor on the membranes of heterologous peritoneal macrophages.

Key words. Complement, pig; complement, system; C3b receptor; β-globulin; macrophages.

It has been suggested that the complement system, especially the third component of complement (C3), is involved in the regulation of such cellular functions as lymphocyte proliferation[1], antibody production[2] and phagocytosis[3]. The C3 probably plays the central role in the activation and functions of the whole complement cascade. By the activation of C3 fragments C3a (anaphylatoxin) and C3b were produced; the latter is capable of binding on the surface of the cell, bacteria, and immune complexes. The C3b mediates immune adherence and thus permits phagocytosis[3,4]. C3 plays a central role in both the classical and alternative pathways[5,6].

The isolation of C3 from the sera of humans[7], cats[8], rats[9], rabbits[10] and pigs[11] has already been described. In this paper the conditions for the isolation and characterization of pig C3 were examined. The isolated pig C3 was used for the detection of the receptor for C3, employing the immunofluorescence technique. The C3 receptors on the macrophage surface are very important structures because of their ability to increase the adsorption of bacteria and other opsonized particles[12].

Materials and methods. The isolation of pig C3 was carried out by the modified method already described[7]. Pig C3 was purified from pooled fresh pig serum by the following sequential steps: PEG 6000 (Lachema, Brno) precipitation 5–12%, DEAE-cellulose chromatography (Reanal, Budapest) with subsequent desalting by Sephadex G-25 (Pharmacia, Uppsala) chromatography, PEG 6000 precipitation to 16%, gel filtration on Sepharose 6B (Pharmacia, Uppsala) and chromatography on hydroxylapatite (prepared by the method of Tiselius et al.[13]). Further ion-exchange chromatography on QAE-Sephadex A-50 equilibrated with 25 mM Tris-HCl buffer (pH 7.8) containing 2 mM EDTA and 100 mM NaCl was used. The column was developed with a linear NaCl gradient to a