ically by plotting $1/v$ versus the concentration of the inhibitor (only competitive inhibitions were indeed found).

Results and discussion. Protein methyltransferase II from human erythrocytes was found to be inhibited by several analogues of S-adenosyl-L-homocysteine (the natural inhibitor of the methylases). All were competitive inhibitors and thus bound to the site normally occupied by S-adenosyl-L-methionine. The results of our investigation have been summarized in the table.

For derivatives 2–5, no inhibition could be observed at concentrations respectively of 30, 600, 160 and 110 μM. As a consequence, the K_i-values were estimated to be greater than 300, 6000, 1600 and 1100 μM. A 80 M solution of derivative 10 decreased by 10% the enzymatic activity. Its inhibition constant was thus estimated to range around 800 μM. More accurate results could not be obtained for these compounds due to their poor solubilities in aqueous solvents.

Examination of the table reveals that S-adenosyl-L-homocysteine remains by far the most effective inhibitor of protein methyltransferase II. Modification at the level of the sulphur atom typified by the conversion into the sulfoxyde results in a decrease of the K_i-value. More important losses of inhibition are observed after the replacement of the amino acid portion of S-adenosyl-L-homocysteine. Most of these compounds so obtained however remain inhibitors, and it may be surmised that all of them would be so if it is taken into account that adenosine itself inhibits the methylase. Thus if the amino acid part of S-adenosyl-L-homocysteine contributes to the binding to the enzyme, a non-negligible contribution of the base and/or ribose moieties is to be awaited. The results and the conclusions obtained here with protein methyltransferase II are different from those published recently concerning a protein (arginine) N-methyltransferase[15]. This enzyme seems indeed to accomodate quite well to any change in the amino acid portion of S-adenosyl-L-homocysteine.

Surprising results have been obtained with derivatives 7 and 12. The binding constant of the latter is to be considered with caution since its methyl donor capacity has not yet been measured (under study).

Derivative 7 was also shown to be a good inhibitor of the protein methyltransferase(s) from *Echerichia coli* responsible for the methylation of the ribosomial protein L 11 of this microorganism (unpublished). It should be pointed out that a good inhibitor of protein methylases may be of practical use for in vitro studies. S-adenosyl-L-homocysteine cannot be utilized for that purpose, being rapidly metabolized in the cells. Finally, it should be emphasized that the pharmacological properties of SIBA (cited in the introduction) do not seem to be related to an inhibition of protein methyltransferase II.

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Inversal relationship between basal adenylcyclase activity and maximal degree of stimulation in membranes of red blood cells from rats[1]

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Summary. The degree of stimulation of adenylcyclase activity, in membranes from immature red blood cells from rats, brought about by isoprenaline, guanylyl-imidodiphosphate and sodium fluoride is strongly dependent on the basal activity of the enzyme. The inversal relationship between basal activity and the maximal degree of stimulation by (—) isoprenaline, shows an apparent seasonal dependence.

It becomes obvious from the results published by numerous authors[2–5]that in membranes of different origin the degree of hormonal stimulation of adenylcyclase (AC) activity, and also the 'basal activity' of the enzyme (i.e. cAMP synthesis in the absence of any stimulant), show extreme variations. Experimental procedures, however, to obtain membrane preparations with constant 'basal AC activities' and constant degrees of hormonal stimulation by standardized methods, were unsuccessful[5,6]. During our recent investigations with membrane preparations from immature red blood cells from rats[7,8], we observed also such extreme variations of basal and stimulated AC activities. Our obser-

vations, however, revealed that there obviously exists a well-defined inversal relationship between 'basal AC activity' and the maximal degree of stimulation brought about by stimulants acting either on β-adrenoceptors or on other subunits of the AC system.

Methods and materials. Male Wistar rats (120–150 g b.wt) were kept for at least 3 days under constant environmental conditions (12 h periods of light and darkness, 23 °C room temperature). Reticulocytosis (about 40–60%) was produced in the animals by treatment with acetyl-phenylhydrazide (40 mg/kg/day i.m. at 10.00 h) on 3 consecutive days. The rats were decapitated and exsanguinated on the 7th day after the first injection at 08.00 h. From the heparinized blood samples, membrane preparations were prepared according to Gauger et al.[9] under strongly controlled conditions. AC activity was measured under suboptimal and also optimal conditions[7,8]. Analysis of variance (one-way) was used for statistical evaluation of the experimental results.

Results and discussion. From the results depicted in figure 1 (obtained in experiments with about 70 different membrane preparations), it becomes obvious that 'basal activities', irrespective of the ion-concentrations used[7,8], showed extreme variations, i.e. by more than a factor of 25. Depending on these basal values, however, are the respective degrees of stimulation: At high basal values, only negligible enhancement of the AC activity could be produced by isoprenaline (Ipn), guanylyl-imidodiphosphate (Gpp(NH)p) or sodium fluoride (NaF) in concentrations known to be maximally effective[8,9]. In contrast, at basal activities lower than 1 nmole cAMP/10 min/mg protein, a more than 10-fold stimulation of AC activity could be achieved.

By variation of certain experimental procedures (modification of the osmolarity of the phosphate buffer, e.g. 0–50 mOsm, used for hypotonic hemolysis, preincubation of the enzyme at temperatures from 4–37 °C, repeated washing of the membranes, storage of the freeze dried membranes at – 20 °C for periods up to 4 weeks) basal and maximally stimulated enzyme activities decreased in parallel. The degree of stimulation therefore remained unchanged.

During a 4 years period using Ipn as a β-adrenoceptor stimulant of AC activity of membranes obtained under identical conditions, we observed that the degree of stimulation was highest during summertime (figure 2, A) when the values of 'basal AC activity' were minimal (figure 2, B). In contrast, relatively low degrees of stimulation could be achieved in membranes prepared especially during wintertime (A) when 'basal activities' were extremely high (B). These results again confirm the inversal relationship between basal and stimulated enzyme activities (figure 1). Furthermore, this inverse correlation shows a seasonal dependence.

Thus, it must be concluded that, in certain membrane preparations, there exists a preformed state of activation which cannot be altered by experimental procedures. It is unlikely that only endogenous catecholamines are responsible for the high 'basal activities' seen in these preparations: The catecholamine concentrations in plasma are far below of their K_a-values in these preparations (10^{-6}–10^{-5} M)[9]. Moreover, pretreatment of rats with Ipn (30 mg/kg, 4 h before decapitation) was without any influence on 'basal activity' (unpublished results). Similar results were also obtained in frog erythrocytes[10]. On the

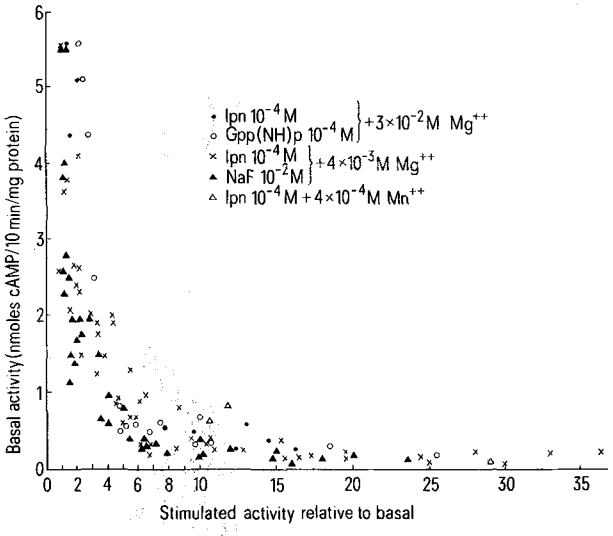

Fig. 1. Inversal relationship between 'basal' (ordinate) and maximally stimulated adenylcyclase activity in membrane preparations from reticulocyte rich blood from rats. The degree of stimulation of enzyme activity is expressed as the ratio 'maximally stimulated activity/basal activity' (abscissa). The enzyme activity was measured in the presence of Mg^{++} at suboptimal (4×10^{-3} M) and optimal (3×10^{-2} M) concentrations and at optimal concentrations of Mn^{++} (4×10^{-4} M)[8]without and with addition of (–)isoprenaline (Ipn), guanylyl-imidodiphosphate (Gpp(NH)p) and sodium fluoride (NaF). Each point represents the mean value from 1 experiment performed in duplicate or triplicate.

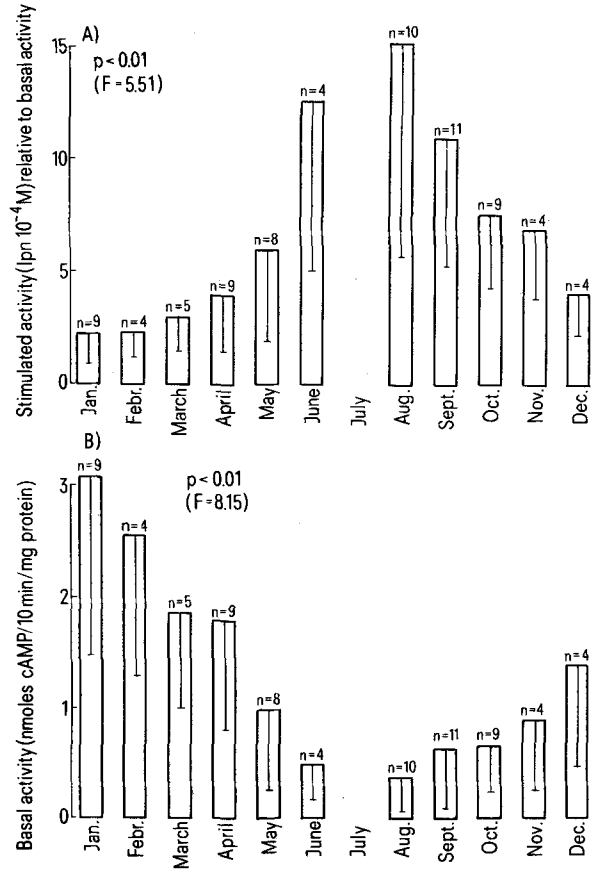

Fig. 2. *A* Seasonal variations of the degree of maximal stimulation of adenylcyclase activity by (–)isoprenaline (10^{-4} M). Ordinate: ratio 'stimulated enzyme activity/basal activity'.
B Seasonal variations of basal activities of adenylcyclase. Columns = $\bar{x} \pm$ SEM from experiments performed in duplicate in n different membrane preparations. The respective single values correspond to those depicted in figure 1.

other hand, AC activity is also subject to allosteric activation[11] by cytosolic factors as well as by nucleotides like GTP[12-15]. If GTPases are inhibited, e.g. by choleratoxin, a highly activated and persisting state of AC activity will be elicited similar to that produced by Gpp(NH)p[16]. Moreover, in certain membrane preparations GTP and low concentrations of catecholamines can activate AC activity in an overadditive manner[14,17]. In some experiments we observed, like other authors[5], a decrease of high basal activity brought about by (±)propranolol (10^{-6} M) up to 50%. From these investigations, it might be speculated that in membrane preparations GTP is bound to a varying degree depending on the respective GTPase activity. When this enzyme activity is low, GTP and small amounts of catecholamines bound to the membranes might elicit highly preactivated states of AC activity which can be stimulated by Ipn, Gpp(NH)p and NaF respectively only to a minor degree. The possibility that other cytosolic factors[15], adherent to the cytoplasmic membrane, were also of influence on the variations of AC activity cannot be excluded.

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Inhibition of α-chymotrypsin by 2-halogeno-ethanols; comparison with 2-methyl-ethanols and urea

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Summary. 2-Halogeno- and 2-methyl-ethanols inhibit α-chymotrypsin in the order of their substituted groups: [1] tri- > di- > mono-, [2] Br- > Cl- > CH_3- > F-. The inhibition by the halogeno-ethanols is mediated differently from that by the methyl-ethanols, ethanol, and urea.

The introduction of a Cl-group instead of the methyl-group on 1-propanol (2-chloroethanol) enhances the ability to change protein conformation as reported by Tanford[1]. However, the effect derived from the change in the substitution number and species of halogeno- groups remains obscure. In this report, we present the effect of 2-halogeno-ethanols (2-fluoroethanol, 2,2,2-trifluoroethanol, 2-chloroethanol, 2,2,2-trichloroethanol, 2-bromoethanol, 2,2,2-tribromoethanol) on the hydrolytic activity of α-chymotrypsin on N-benzoyl-L-tyrosine-p-nitroanilide[2] and comparing them with 2-methyl-ethanols (1-propanol, 2-methyl-1-propanol, 2,2-dimethyl-1-propanol), ethanol, and urea.

Materials and methods. α-Chymotrypsin (bovine pancreas) and N-benzoyl-L-tyrosine-p-nitroanilide were obtained from Sigma, St. Louis, ethanol, 1-propanol, 2-methyl-1-propanol, 2,2-dimethyl-1-propanol, 2-fluoroethanol, 2-chloroethanol, 2,2,2-trichloroethanol, 2-bromoethanol from Wako, Osaka, and 2,2,2-trifluoroethanol and 2,2,2-tribromoethanol from E. Merck, Darmstadt.

Enzyme assay. The enzyme reaction was performed at 30 °C in 4 ml of a reaction mixture (pH 8.0) consisting of 1.9 ml of substrate-buffer (1 vol. of 1.25 mM N-benzoyl-L-tyrosine-p-nitroanilide in ethanol plus 4 vols of 0.1 M Tris-HCl, pH 8.0, 0.01 M $CaCl_2$), 2 ml of deionized water (a control) or 2 ml of aqueous solutions of urea or alcohols that are soluble in water in any proportion (A series: urea, ethanol, 1-propanol, 2-fluoroethanol, 2,2,2-trifluoroethanol, 2-chloroethanol, 2-bromoethanol) or 2 ml of 5 M ethanolic solutions of the alcohols, including some that are hardly soluble in pure water (B series: ethanol, 1-propanol, 2-methyl-1-propanol, 2,2-dimethyl-1-propanol, 2-fluoroethanol, 2,2,2-trifluoroethanol, 2-chloroethanol, 2,2,2-trichloroethanol, 2-bromoethanol, 2,2,2-tribromoethanol), and 0.1 ml of α-chymotrypsin in 0.001 N HCl (553 μg/ml). The p-nitroaniline liberated was determined spectrophotometrically at 400 nm.

Results and discussion. The p-nitroaniline liberated in 5 min by the α-chymotrypsin in the absence of urea and the alcohols (a control, 0.0825 μmoles) and in the presence of urea or the alcohols are given as function of the urea and alcohols concentration in figure 1.

In series A, urea in any concentration inhibited the enzyme. Ethanol in concentrations below 1 M had no effect on activity, and in higher concentrations inhibited the enzyme. These reagents caused no activation, but all the substituted ethanols in series A caused perceptible activation at low concentrations, whereas at high concentrations they inhibited the enzyme. Urea, which has no hydrophobic group, destabilizes native conformations by negative free energies of transfer from water to urea of hydrophobic groups[3-5] and polar groups[6]. Therefore, though it is not evident, it is possible that the different profiles in series A (urea and ethanol, inhibition; substituted ethanols, activation to inhibition) resulted from their different ability to invade the hydrophobic regions in the enzyme. The inhibition