may contribute to terminating the action of the putative neurotransmitter glycine. The significance of the postsynaptic localization of sarcosine in the squid giant synapse is unknown.

Examination of previously published dansyl chromatograms show sarcosine is restricted to the brain in *Helix*[12] tissues and present in the blood[10]. It is unaffected by electrical stimulation of the brain[18], or by treatment with LSD-25[11]. Our own and earlier comparative studies show it to be the highest in crustaceans and cephalopods[19]; but absent from the leech segmental nerve chain[20], dorsal root ganglia[6], and the optic pathway[2,5].

Summary. An unknown dansyl derivative was identified as dansyl sarcosine. In molluscs, sarcosine was found to be largely localized to the nervous system. Examination of individual snail neurones, regions of the octopus brain and the squid giant synapse showed dramatic variations in sarcosine levels.

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Effect of Some Phosphodiesterase Inhibitors on Two Different Preparations of Adenosine 3′,5′-Monophosphate Phosphodiesterase

Many cellular functions are regulated by adenosine 3′,5′-cyclic monophosphate (cyclic AMP). The intracellular concentration of cyclic AMP is generally under hormonal control. Therefore it seems of special interest to study the effect of chemicals, especially therapeutic agents, on the regulation of cyclic AMP in in vitro systems and in intact cells.

Cyclic AMP phosphodiesterase (PDE), the enzyme which degrades cyclic AMP to 5′-AMP, represents a possible target for influencing the intracellular concentration of cyclic AMP by chemical means. When compounds are tested for their activity as inhibitors of PDE, often the concentration of the compound necessary to inhibit PDE activity by 50% (I_{50}) is determined[1-3].

PDE seems to exist in a variety of isoenzymic forms which may occur simultaneously in a particular tissue[4-6]. To evaluate the potency of a compound as PDE inhibitor in a specific tissue, it seems desirable to measure its PDE inhibiting activity on more than one preparation of PDE. Such a study, using two different PDE preparations, is presented in this paper.

Materials and methods. Two different PDE preparations from rat brain cortex were used. One was prepared according to KAKIUCHI[7] which included Sephadex G-200 column chromatography of a supernatant obtained after centrifugation of the homogenate of rat brain cortex. The Sephadex G-200 column chromatography yielded 3 fractions with PDE activity. One fraction showed enzymatic activity only in the presence of modulator protein and calcium ions. This fraction was used further and will be designated PDE I. Modulator protein was prepared by heating a hog brain cortex homogenate for 5 min at 95 °C in a water bath[7].

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I_{50} values of some PDE inhibitors using two different PDE preparations and two different substrate concentrations

Substrate	Concentration (μM)	IBMX I_{50} (μM)	Papaverin I_{50} (μM)	SQ66-007 I_{50} (μM)	SQ20-009 I_{50} (μM)	n
PDE I	500	9.25 ± 4.3	48.5 ± 19	35.1 ± 9.7	28.5 ± 4.6	4
PDE I	0.25–2	24.6 ± 5.3	94.4 ± 3.7	64 ± 10.8	n.d.	4
PDE II	500	67.75 ± 6.1[b]	52.87 ± 5.5 n.s.	56.5 ± 10.1[a]	n.d.	5
PDE II	0.25–2	44.2 ± 5.4[b]	33.7 ± 19.6[b]	19.1 ± 4.6[b]	n.d.	5
PDE I	500	$2p < 0.01$[c]	$2p < 0.01$[c]	$2p < 0.01$[c]		
PDE I	0.25–2					
PDE II	500	$2p < 0.001$[c]	n.s.[c]	$2p < 0.001$[c]		
PDE II	0.25–2					

The incubation mixture contained in a total volume of 1.0 ml; 0.2 mM dithiothreitol; 1 mM 5′-AMP; 3 mM $MgSO_4$; 0.5 mM ^{3}H 3′,5′-cyclic AMP (60 nCi); 0.1 mM $CaCl_2$; varying concentrations of inhibitor and 36 µg of PDE I plus 71 µg modulator protein or 420 µg PDE II. The reaction was stopped by addition of 0.2 ml $ZnSO_4$ (0.2 mM) and 0.2 ml $Ba(OH)_2$ (0.2 mM). After centrifugation, radioactivity of the supernatant was determined. [a] < 0.05, [b] < 0.001 as compared to PDE I. n.s. not significant, [c] Iso values at high substrate concentration compared to those at low substrate concentration: n.d., not determined. IBMX: Isobutylmethylxantine. All values represent mean ± S.D.

Another PDE was prepared according to CHASIN[8], essentially being the ammoniumsulfate fraction from 0–50% saturation of the crude homogenate of rat brain cortex. This fraction was collected by centrifugation. The pellet was dissolved in a small volume, dialyzed and used without further purification. This preparation will be designated PDE II.

Inhibitors of PDE were tested on these PDE preparations, using a substrate concentration of 500 μM and of 0.25–2 μM. PDE was routinely assayed according to the procedure described by PÖCH[9] with minor modifications. In this assay, 1 mM 5′-AMP was included in the incubation mixture to trap the radioactive product.

When the two-step method of BUTCHER[10] was used to measure PDE activity, the results obtained were identical. When chemicals were tested for their ability as PDE inhibitors, a preincubation period of 10 min with the compound at 30 °C preceded the start of the enzymic reaction with cyclic AMP. Assays were carried out in duplicate and mean values were calculated. Statistical comparisons of data were made by using Student's t-test. Protein was determined by the method of LOWRY[11].

(8-^{3}H) adenosine 3′,5′-cyclic monophosphate was from Amersham (27.5 Ci/mmol). Isobutylmethylxanthine was a gift from Searle, Chicago; chlordiazepoxide, diazepam and RO 20–1724 were a gift from Hoffmann-La Roche; SQ 20–009 and SQ 66–007 were a gift from Squibb, Princeton N. J. RO 20–1724: 4-(3-butoxy-4-methoxybenzyl-)2-imidazolidinone. SQ 20–009: 1-ethyl-4-(isopropylidene hydrazino)-1 H-pyrazolo (3,4-b) pyridine-5-carboxylic acid, ethylester, hydrochlorid. SQ 66–007: 5-acetyl-4-(sec. butylamino-)-1-ethyl-1 H-pyrazolo-(3,4-b)pyridine.

Results. A total of 9 compounds were tested as inhibitors of the PDE's. Using PDE I, diazepam and chlordiazepoxide had I_{50} values of 100 μM at 500 μM cyclic AMP concentration. At this substrate concentration papaverine, SQ 66–007, SQ 20–009 and isobutylmethylxanthine were inhibiting the PDE I, isobutylmethylxanthine being the most potent, $I_{50} = 9.25 \pm 4.3$ (Table). Using cyclic AMP at a concentration of 0.25–2 μM with PDE I the order of potency of the latter compounds did not change; however, the I_{50} values of the inhibitors were significantly increased compared with the values obtained with the high substrate concentration (Table).

When PDE II was used as the source of enzyme, inhibition of the enzymatic activity by 50% did require significantly higher concentrations of isobutylmethylxanthine and SQ 66–007 (500 μM cyclic AMP as substrate) compared to the values obtained with PDE I. Again, when the lower concentration of substrate cyclic AMP was used, the I_{50} values were significantly different from the I_{50} values at the high substrate concentration (Table).

Theophyllin, RO 20–1724 and clonazepam at 5–80 μM did not inhibit hydrolysis of cyclic AMP at 500 μM substrate concentration by either PDE I or PDE II. Using low substrate concentrations, PDE I and PDE II showed also different K_m values, 2.5 μM and 20 μM respectively (Figure).

Discussion. There is a tendency to relate PDE-inhibiting properties of chemicals to their pharmacological effects[1–3,12]. If this were possible, I_{50} values should be fairly constant when different PDE preparations are used as enzyme sources. Our results show that it is very difficult to correlate the potency of a drug as inhibitor of PDE with its pharmacological mechanism of action. Depending on the kind of PDE and the substrate concentration used in the test system, a drug may be a potent or rather impotent inhibitor of PDE.

In contrast to our knowledge about the various PDE isoenzymes isolated from tissues and clonal cell lines, little is known about the form (s) of PDE (s) which exist in intact cells. Estimates of the actual cyclic AMP concentration at the site of PDE within the cell are almost impossible. Our data show that these parameters may strongly influence the activity of a given drug to act as inhibitor of PDE. More detailed studies particularly on the actual forms of PDE existing in unbroken cells are necessary.

Zusammenfassung. Neun Substanzen wurden an zwei verschiedenen PDE Präparationen auf ihre PDE hemmenden Eigenschaften untersucht. Dabei zeigte sich, dass die I_{50}-Werte von der Art der Präparation und von der Substratkonzentration abhängen.

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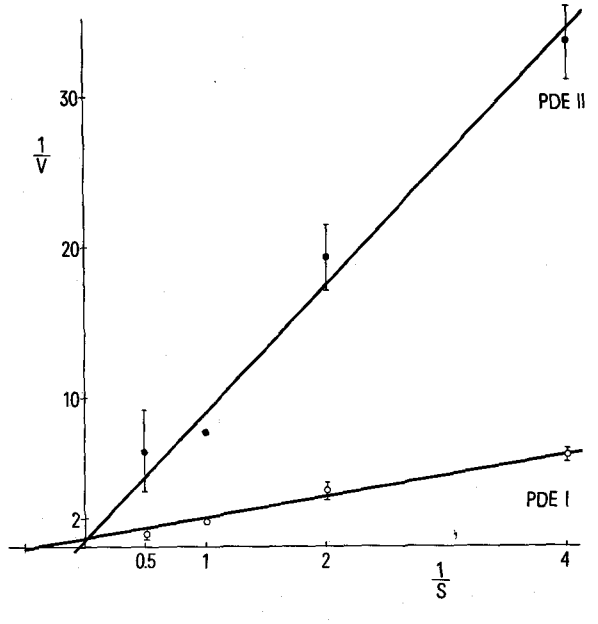

Lineweaver-Burk plot of cyclic AMP hydrolysis by PDE I and PDE II. Assay was performed as described in legend to the Table. Lines were best fitted by means of a computer program. V is expressed as: μmoles cyclic AMP hydrolyzed/mg protein × min.
All values represent mean ± S.D. (n = 3).

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