amylase (figures 1 and 2) and lipase (figure 2) is immediate and sustained (figure 1) after a 30 min preincubation of the pancreatic fragments in presence of VB 5×10^{-5} M. Increased enzyme release by glands treated with DbcAMP 1 mM alone or in association with VB 5×10^{-5} M is confirmed by the observation of numerous pictures of exocytosis at the apex of the acinar cells as well as the presence of a dense secretory material in the acinar lumina.

DbcGMP 1 mM and VB 5×10^{-5} M, combined or not, do not significantly affect the spontaneous release of enzymes from pancreas in vitro (figures 1 and 2). In addition, DbcGMP associated with VB or alone does not modify the secretory response to DbcAMP (figures 1 and 2). These data are confirmed by the failure of DbcGMP to induce, by itself, any ultrastructural modifications.

Discussion. In a previous report[18], it was suggested that DbcAMP stimulates enzyme release from the pancreas through a direct interaction with the acinar cell. The present findings support and strenghten this view and imply the participation of microtubules in the secretory process induced by the nucleotide derivative. Indeed, the disappearance of microtubules in the acinar cell, depending on the concentration and the time of exposure to the vinca alcaloid, is accompanied by an increase of enzyme secretion in response to DbcAMP. However, the intracellular mechanism of action remains unclear. Considering the findings of Haymovits and Scheele[5] that the cellular level of cGMP is enhanced by DbcAMP, it may be speculated that cGMP plays a major role in modulating the dynamic equilibrium between microtubules and their subunits. But the microtubule-inhibitor does not affect the secretion of enzymes in presence of the dibutyryl derivative of cGMP. Moreover, the latter is completely inert on the structure and function of the exocrine pancreas, as already noted by Heisler and Grondin[13]. While the impermeability of cell membranes to this nucleotide derivative cannot be disregarded, further investigations may be necessary to clarify the interaction between cyclic nucleotides and microtubules in the pancreatic acinar cell.

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'High-affinity' binding sites for glycine in synaptosomal-mitochondrial fractions of rat CNS regions[1]

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Summary. 'High-affinity' binding mechanisms for glycine exist in synaptosome-enriched preparations of various regions of rat CNS. Such mechanisms may represent interactions of glycine with its synaptic receptors.

Glycine may be a post-synaptic inhibitory neurotransmitter in the vertebrate CNS[2-6]. 'High-affinity' mechanisms for the binding and uptake of glycine to slices and subcellular particles of various CNS regions have been demonstrated[7-10]; but such mechanisms do not appear to involve an association of glycine with synaptic receptors. However, Young and Snyder[11] have shown that ^{3}H-strychnine is bound to crude CNS membrane preparations by a 'high-affinity' mechanism ($K_D \cong 3 \times 10^{-9}$ M) which might represent an interaction of this drug with glycine-receptors. The present study reveals that 'high-affinity' glycine-binding mechanisms, which could be related to synaptic receptors, exist in 'synaptosomal-mitochondrial' fractions of several regions of rat CNS.

Materials and methods. Wistar rats weighing 175–360 g (males for data of figures 1 and 2 and females for data of the table) were decapitated, and the following CNS regions were rapidly excised and pooled: cerebral cortex (2 rats); whole cerebellum (3 rats); cervico-thoracic spinal

Amino acid competition for specific ^{3}H-glycine binding sites

Amino acid (10^{-3} M)	^{3}H-Glycine bound (pmole/g P_2) Total	Specific	Decrease in specific ^{3}H-glycine binding (%)
None	179.3 ± 14.2	159.8	–
Glycine	19.5 ± 1.9	–	100
β-Alanine	112.8 ± 10.4	93.3	41.6
L-α-alanine	120.8 ± 10.6	101.3	36.6
DL-β-aminoisobutyric acid	131.8 ± 14.4	112.3	29.7
Taurine	157.3 ± 16.8	137.8	13.8
GABA	160.2 ± 14.8	140.7	12.0
L-Glutamate	163.2 ± 17.2	143.7	10.1

^{3}H-glycine concentration was 2.4×10^{-8} M; pellets were corrected for trapped supernatant fluid using sucrose distribution ratios[12]. Specific ^{3}H-glycine binding was obtained by correcting values for 'nonspecific' binding which occurred in the presence of 10^{-3} M unlabelled glycine. Means ± SEM or mean values; 6 samples in all cases.

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cord, medulla oblongata, pons and midbrain (each from 5 or 6 rats). In some experiments (table), cervico-thoracic spinal cord, medulla oblongata, pons and midbrain were pooled.

Tissues were homogenized in 10 vol. isosmotic (0.32 OsM) sucrose solution, and 'synaptosomal-mitochondrial' (P_2) fractions were prepared[12]. P_2 fractions were resuspended in 3.0 ml of bicarbonate-buffered physiological medium[13], 0.25-ml aliquots being distributed to previously tared centrifuge tubes. Then, 0.2 ml of physiological medium, either free of added substances or providing final concentrations of 10^{-3} M unlabelled glycine or other amino acids, or 10^{-9}–10^{-3} M strychnine-SO_4, were added. After agitating the samples and allowing them to stand at 0 °C for 10–15 min, 0.5 ml of physiological solution providing $4.7–47 \times 10^{-9}$ M ^{3}H-glycine (2-^{3}H-glycine; New England Nuclear Corp; 11.1 Ci/mmole)-plus-1.7 — 17.4×10^{-8} M ^{14}C-sucrose (U-^{14}C-sucrose; Amersham, England; 610 mCi per mmole) was added to each preparation. After mix-

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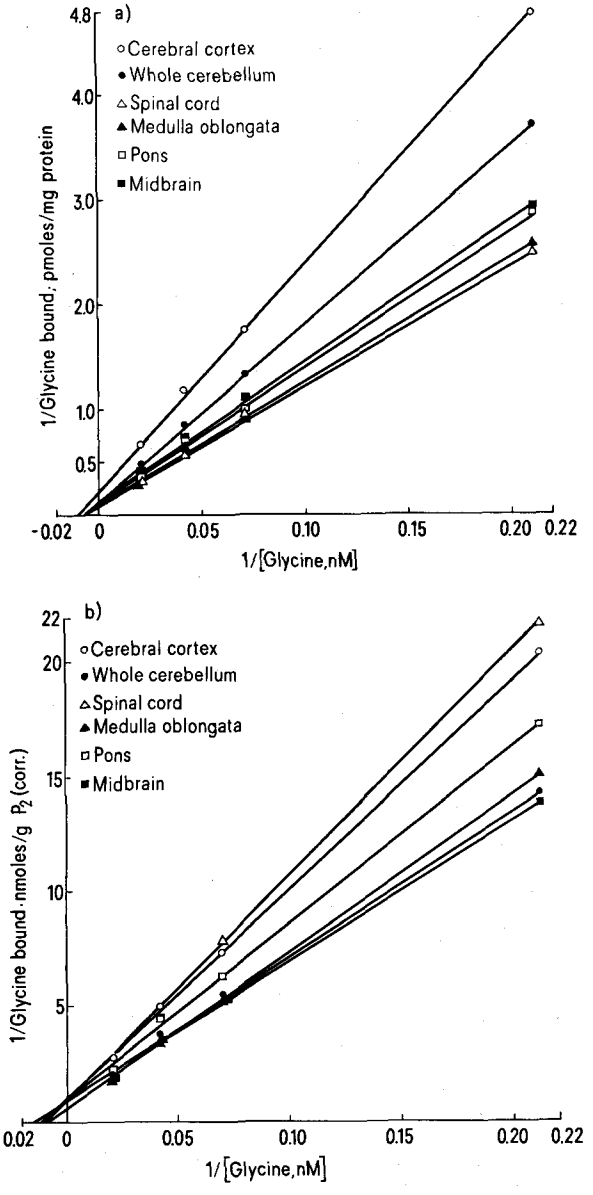

Fig. 1. Lineweaver-Burk plots for the 'high-affinity' binding of ^{3}H-glycine to 'synaptosomal-mitochondrial' fractions of regions of rat CNS. Values are expressed as mole ^{3}H-glycine/mg protein (a) and as mole ^{3}H-glycine/g P_2, corrected for sucrose space (b). K_D values obtained from these plots ranged from about $0.8–1.6 \times 10^{-7}$ M, and corresponding B_{max} values ranged from 4.7–11 pmoles/mg protein (a) or from 1.1–2.3 nmoles/g P_2 (b). All points are means of 4–6 samples, individual values varying less than 15%.

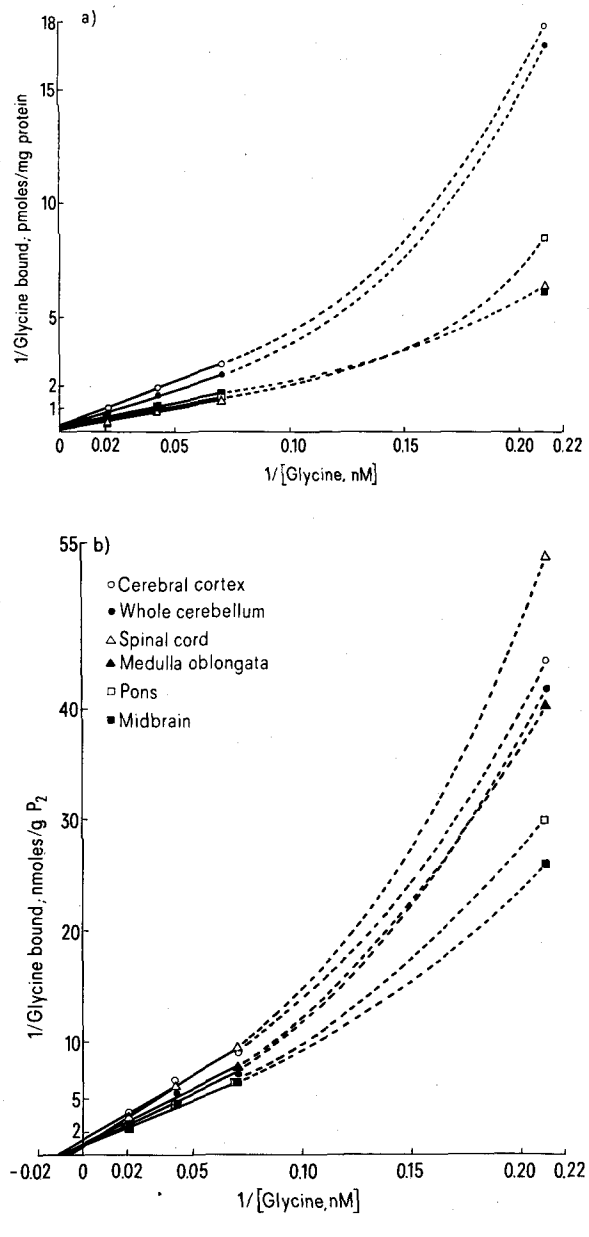

Fig. 2. Lineweaver-Burk plots of the 'high-affinity' binding of ^{3}H-glycine to 'synaptosomal-mitochondrial' fractions of regions of rat CNS, after correcting the data for 'nonspecific' ^{3}H-glycine-binding which occurred in the presence of 10^{-3} M unlabelled glycine. Note that these parabolic functions display upward concavities, indicating that positive cooperativity is involved in the interaction of glycine with its receptors. Mean values; 4–6 samples in all cases; individual values varied less than 15%.

ing the samples and allowing them to stand for another 10–15 min, they were centrifuged at 17.000 × g, 30 min, 0 °C, to prepare final pellet and supernatant fractions. Radioactivity due to ^{3}H and ^{14}C was determined by a previously-described method[12] and protein was determined by the method of Lowry et al.[14]. ^{14}C-sucrose distribution ratios provided measures of the supernatant fluid in the pellets. The data are expressed as mole ^{3}H-glycine bound/mg protein and as mole ^{3}H-glycine bound/g P_2, corrected for sucrose space[12]. Values were corrected further for 'nonspecific' binding of ^{3}H-glycine that occurred in the presence of 10^{-3} M unlabelled glycine[11].

Results and discussion. 'High-affinity' mechanisms for ^{3}H-glycine binding to 'synaptosomal-mitochondrial' fractions of 6 regions of rat CNS were evident in data presented on a protein basis (figure 1a) and on a weight basis (figure 1b). Corresponding K_D values were similar for all regions, and ranged from about $0.8–1.6 \times 10^{-7}$ M. It is noteworthy that the order of potency of these binding mechanisms varied with respect to both the region analyzed and the method used for expressing the data. Correction of the data for the amounts of ^{3}H-glycine bound in the presence of 10^{-3} M unlabelled glycine revealed that these binding mechanisms involved positive cooperativity. This is evident from the parabolic, concave-upward Lineweaver-Burk plots shown in figure 2. Hill plots of these data possessed slopes that ranged from about 1.25–1.50. These results support the observation of Werman[15,16] that more than one glycine molecule is necessary for activation of glycine-receptors. Such cooperativity is also in accord with the finding of Giambalvo and Rosenberg[17] that GABA binding to post-junctional complexes of rat cerebellum occurred with a Hill coefficient of about 2.2 and with physiological results which have indicated that 2 GABA molecules are required to activate the GABA-receptor of the crayfish neuromuscular junction[18]. Results shown in the table revealed further that competition of several amino acids for these ^{3}H-glycine-binding sites paralleled their relative potencies at mimicking the post-synaptic depressant action of glycine[2,19], but not their relative potencies at inhibiting glycine-binding to CNS transport-receptors; e.g., β-alanine exerts an effect similar to that of glycine on spinal neurones[19], whereas it does not compete significantly with glycine for binding to CNS membrane fragments[10]. As observed previously with higher concentrations of glycine[20], this binding was sensitive only to $10^{-5}–10^{-3}$ M strychnine-SO_4 (data not shown), indicating that glycine and strychnine are bound to distinct CNS sites.

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Tolerance to cold and glucose homeostasis in adrenal demedullated dogs[1]

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Summary. The rise in O_2 consumption and in glucose turnover, induced by acute cold exposure is not suppressed by adrenal demedullation in dogs. However, both at neutral and cold ambient temperature, the mean plasma glucose concentrations are higher in normal (N) than in adrenal-demedullated dogs (ADMX). In the cold, the fall in rectal temperature is larger in ADMX than in N dogs.

When acutely exposed to low ambient temperatures, dogs are able to increase their metabolic rates up to 10 times the BMR[3]. Such an energy expenditure is covered by a parallel increase in substrates supply, mainly FFA and glucose. Previous experiments conducted in cold-exposed normal dogs have shown that there is an almost linear relationship between glucose turnover, as measured by U-^{14}C-glucose, and energy expenditure for O_2 consumption, ranging from 5 to 40 $ml \cdot kg^{-1} \cdot min^{-1}$ [4]. In addition, no significant drop in plasma glucose concentration has been observed in these dogs, even at times when they were exposed for more than 3 h to ambient temperatures far below − 20 °C. This seems to suggest a rather strict balance between glucose production and utilization. These results prompted us to look for the mechanisms permitting glucose metabolism to be so perfectly adapted to the requirements of energy needs. Adrenomedullary secretion is increased during cold exposure[5], and the hyperglycemic effect of catecholamines is well-documented (for review, see Himms-Hagen[6]). The aim of this investigation was to compare energy expenditure, tolerance to cold and glucose turnover in normal and adrenal-demedullated dogs exposed to neutral and cold ambient temperatures.

Methods. 16 unanesthetized adult female mongrel dogs, with body weights ranging from 7.8 to 16.1 kg (mean: 11.3 kg) were used in 49 experiments. They were housed in a + 22 °C temperature-controlled room and fed about 300 g of a dry commercial pet food (U.A.R. 121, containing 42.5% of its calories in carbohydrate form). The animals were fed daily between 17.00 h and 18.00 h, before the experiment on the following day, so that the period of fasting was about 15 h at the beginning and 19 h at the end of the experiment. They were given tap water ad libitum. 10 dogs were used in 18 experiments

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