Table II.

Age (months)	D_c	Thk_c (from Table Ia)	Thk_{Pb}	D_{Pb} theo-retical	D_{Pb} ob-served	Δ obs.–th.
1	31.4	5.96	6.06	30.37	36.26	+ 5.88
2	35.3	5.67	4.86	48.04	41.91	− 6.13
3	31.48	5.61	4.38	51.64	40.76	−10.88

Abbreviations: D_c and D_{Pb}: mean capillary densities in control and leadtreated animals. Thk_c and Thk_{Pb}: mean cortex thickness. Δ: difference between observed and predicted capillary density values after lead treatment.

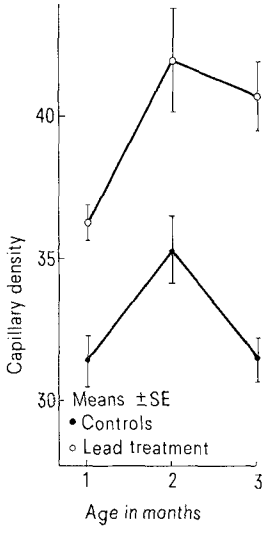

Fig. 2. Influence of age on the capillary density. The capillary density values correspond to type I measurements of Table Ia.

The cerebral cortex of the lead-treated animals in this light microscopic study does not display evident qualitative lesions. Vascular strands are, however, more frequent than in control animals, although the lead dose was lower than that used by other investigators[3,10,11]. Contrary to CLASEN[11], we consider these vascular strands as tangential sections of capillaries: indeed, their number can be considered as proportional to the characteristic high convolution factor seen in lead-treated animals (Table Ic). Moreover in the majority of cases, the use of 3 µm thick sections makes it possible to discern the presence of vascular lumina, which evidently occur more rarely in ultra-thin sections destined to electron microscopy.

In addition, the capillaries in lead-treated brain are not only more convoluted but also more numerous than in the controls. A possible reason for the enhancement of small vessels density and convolution is the significant reduction in thickness of the cortex in lead-treated rats (Table Id). Nevertheless, other factors may play a role also for the following reasons: 1. the involution of the brain is not yet noticeable in the 1-month-old animals (at the end of the suckling period) but still, their capillary density is already significantly increased. 2. Although the capillary density is much enhanced, it is lower than to be expected from the reduction in cortical size. Such a prediction was made on the assumption that as the thickness of the cortex decreases, the density of the vascular supply would increase according to a simple equilateral hyperbolic function: $D_{Pb} = \frac{Thk_c^2 \times D_c}{Thk_{Pb}^2}$ where D_{Pb} is the evaluated capillary density for the lead-treated animals; Thk_c and Thk_{Pb} the thickness of the cerebral cortex in both kinds of animals; D_c the observed density of capillaries in controls. However, the capillary density observed in lead-treated rats was consistantly lower than the predicted one (D_{Pb}) and this difference (Δ in Table II) increased with time of lead treatment. The difference may indicate that the disturbed relationships between nervous and vascular components in the cerebral cortex begin to readjust slowly according with the time elapsed. The present data would thus suggest that lead acts primarily on the grey matter and that the quantitative and conformational changes in the vascular supply represent mainly a sequel of this effect.

Whatever the case, the vulnerability of brain blood vessels to lead still deserves further investigation, principally at low dose levels and electron microscopy studies, using the same material, have now been brought to bear on this particular problem of the equilibrium relations between vascular, glial and nervous elements in the lead-treated cerebral cortex of the infant rat.

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Adenosine Promoted Accumulation of Adenosine 3′,5′-Monophosphate in Rabbit Vagus Nerve

P. ROCH and A. SALAMIN[1]

Département de Pharmacologie, 20, Ecole de Médecine, CH–1211 Genève 4 (Switzerland), 11 May 1976.

Summary. Desheathed rabbit vagus nerve has been found to form cyclic AMP when incubated with adenosine. This accumulation of cyclic AMP is inhibited by theophylline but not by antiadrenergic agents, anticholinergic agents or local anaesthetics. Depolarizing media are not able to promote cyclic AMP accumulation in this preparation.

Nervous tissues synthesize adenosine 3′,5′-monophosphate (cyclic AMP) under various conditions[2–5]. In peripheral nervous tissue, attention has been focused on sympathetic ganglia, especially the superior cervical ganglion, where cyclic AMP accumulation has been related to the formation of the slow inhibitory postsynaptic potential[6]. Tests on peripheral nerve have shown only an absence of cyclic AMP accumulation in response to electrical stimulation[7]. Such a negative result, however, is not sufficient to conclude that peripheral axons do not accumulate cyclic AMP. It is of particular importance to re-investigate this possibility, because of

the much greater simplicity of peripheral nerve in comparison with central nervous tissue, or with sympathetic ganglia. The simplicity is essentially due to the lack of synapses. Since, in brain, it appears that there are several compartments of adenylate cyclase[8], it would be of great interest to find a non-synaptic adenylate cyclase in nervous tissue.

As a first step, we have investigated the action of depolarizing agents and adenosine on cyclic AMP concentrations in the rabbit cervical vagus nerve, a preparation which consists mostly of small non-myelinated axons[9].

Material and methods. Vagus nerves were rapidly removed from shot rabbits weighing between 2.5 and 3 kg. The nerves were carefully desheathed using fine dissecting scissors and divided into 3 parts. The pieces of nerve were pre-incubated, at 37 °C, in Locke for 30 min, or for 15 min in Locke, and 15 min in Locke containing inhibitors. Each piece was then transferred to 5 ml of Locke at 37 °C, containing the substances to be tested. The incubation was stopped by homogenization in ice-cold ethanol containing 0.2 *N* HCl. Insoluble material was precipitated by low-speed centrifugation and then respun in 1 *N* NaOH for protein determination. The supernatants were dessicated under N_2 stream and used for cyclic AMP dosage.

Proteins were assayed by the method of LOWRY et al.[10]. The extinction was read at 600 nm on a Metrohm E 1009 spectrocolorimeter.

For cyclic AMP determination, the dessicated supernatants were resuspended in *Tris*-HCl buffer, 100 m*M*, pH 7.4, containing theophylline, 8 m*M* and 2-mercaptoethanol, 6 m*M*. Cyclic AMP was then assayed by the saturation binding method[11].

Locke contained NaCl, 154 m*M*; KCl, 5.6 m*M*; $CaCl_2$, 0.9 m*M*; $MgCl_2$, 0.5 m*M*; morpholinopropane sulfonic acid (MOPS), 10 m*M*; glucose, 5 m*M*; NaH_2PO_4 and Na_2HPO_4, 0.6 m*M*; pH 7.4. High K, 100 m*M*, Locke was obtained by replacing NaCl with KCl.

Adenosine, atropine sulphate and theophylline were obtained from E. Merck AG (Darmstadt, Germany); cocaine hydrochloride and tetracaine from Siegfried (Zofingen, Switzerland); veratridine from Aldrich (Milwaukee, USA); tetrodotoxin from Sankyo (Tokyo, Japan); phentolamine (Regitin®) from Ciba-Geigy (Basle, Switzerland); haloperidol from Cilag-Chemie (Schaffhausen, Switzerland); sotalol hydrochloride from Mead Johnson and Co. Evansville, Ind., USA); and hexamethonium chloride dihydrate from Schwartz-Mann Biochemicals (Orangeburg, N. J., USA).

Results are expressed as the mean of several independent experiments ± SEM.

Results. a) *Basal value.* Each piece of nerve contained between 0.11 and 0.77 mg of proteins. The cyclic AMP content of these pieces, after pre-incubation for 30 min in Locke at 37 °C was 32.8 ± 2.2 pmol per mg protein ($n = 62$). There was a fairly large variation in basal cyclic AMP concentrations from one experiment to another. Because of this, the experimental values of cyclic AMP concentration are referred to control values in the same experiment and expressed as percentages.

b) *Effect of adenosine.* We obtained an accumulation of cyclic AMP in rabbit vagus nerve by application of adenosine. Figure 1 shows that the accumulation is rapid, reaching a maximal level after 3 min incubation. For this reason, and for technical convenience, we used a time of 6 min for all incubations in the experiments reported here, except for those of Figure 1.

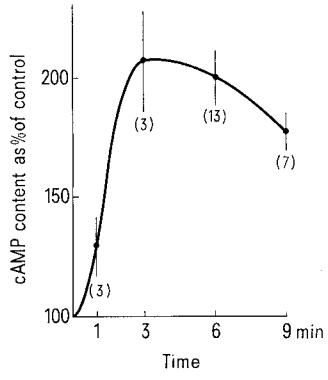

Fig. 1. Time course of the accumulation of cyclic AMP by 100 μ*M* adenosine in rabbit vagus nerve. Results are expressed as percent of unstimulated controls ± SEM. Number of experiments in brackets

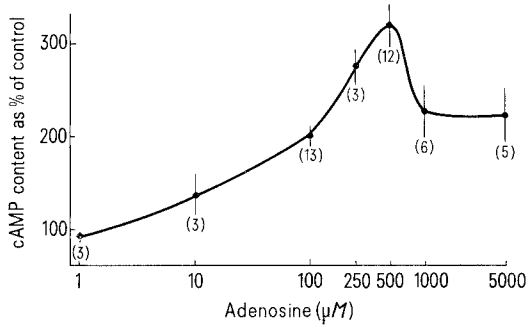

Fig. 2. Logarithmic dose-response curve of cyclic AMP accumulation in rabbit vagus nerve by adenosine. Results are expressed as in Figure 1.

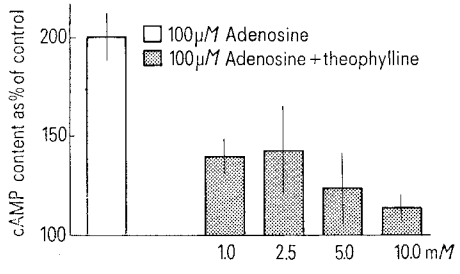

Fig. 3. Inhibition of adenosine elicited accumulation of cyclic AMP by theophylline. 1st column: cyclic AMP accumulation by 100 μ*M* adenosine. 2nd to 5th column: inhibition of the effect of adenosine by various concentrations of theophylline. Pieces of nerves were pre-incubated 30 min in Locke and then transferred to Locke containing 100 μ*M* adenosine and theophylline for 6 min.

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The accumulation of cyclic AMP is dependent on the concentration of adenosine applied. The effect is a maximum at an adenosine concentration of 500 μM; it stabilizes at 1 mM and above, with an increase of cyclic AMP to 230% of controls (Figure 2). A concentration of 100 μM adenosine was used for all further experiments.

c) *Inhibition.* The accumulation of cyclic AMP by adenosine can be inhibited by theophylline. The inhibition is partial with 1 mM theophylline and complete with 10 mM (Figure 3).

We have applied a large number of substances, which are known to inhibit the effect of different agents on adenylate cyclase in other systems. These included α-adrenergic (phentolamine, 0.1 mM), β-adrenergic (sotalol, 0.1 mM), dopaminergic (haloperidol, 0.05 mM), muscarinic (atropine, 0.05 mM), nicotinic (hexamethonium, 0.5 mM) inhibiting agents, as well as local anaesthetics (cocaine, 1 mM; tetracaine, 1 mM and tetrodotoxin, 0.003 mM). None of these substances inhibited the increase in cyclic AMP caused by adenosine.

d) *Depolarizing agents.* Since adenosine is thought to be an intermediary between depolarization and cyclic AMP accumulation in brain[12], the effect of depolarizing agents was tested in the vagus nerve. Pieces of nerve were incubated in presence of high potassium (100 mM) or veratridine (500 μM), which are known to depolarize frog nerve fibres[13] and rabbit vagus nerve[14]. Neither of these two agents produced an increase in cyclic AMP.

Discussion. Our experiments show that there is a very marked accumulation of cyclic AMP when the rabbit vagus nerve is exposed to adenosine. As in guinea-pig brain slices[15], the effect of adenosine is inhibited in the presence of a xanthine derivative, theophylline. It appears that the adenylate cyclase response to adenosine in the vagus nerve is direct and specific, since it is unaffected by agents which, in other tissues, inhibit the adenylate cyclase response to catecholamines, cholinomimetics, or depolarizing agents.

In guinea-pig brain, cyclic AMP content is increased by exposure to depolarizing agents, or to adenosine, and it is thought that adenosine is the intermediary between depolarization and cyclic AMP accumulation[12]. In bovine superior cervical ganglion, depolarizing agents, but not adenosine, produce an accumulation of cyclic AMP[16,17], and it is thought that the intermediary between depolarization and cyclic AMP accumulation is at least partially catecholamines. We have also tested rabbit superior cervical ganglions for their response to adenosine. No significant accumulation of cyclic AMP was found (121 $\pm$ 11% of unstimulated controls, $n = 4$, 100 μM adenosine).

In vagus nerve, only adenosine has an effect on cyclic AMP content. The absence of any effect of depolarization in vagus nerve reveals either the absence of an endogenous adenosine releasing system, or a difference in function of the adenosine-sensitive adenylate cyclases of nerve axons and of central nervous system. It is not known whether cyclic AMP accumulates in the axon or in supporting tissue (Schwann cells).

It will be of interest to investigate further the adenylate cyclase in vagus nerve in order to elucidate the seemingly complex functions of cyclic AMP in the nervous system.

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The Loss of Biological Activity of 5-Hydroxytryptamine Creatinine Sulphate

T. Dalton

Department of Zoology, Westfield College, Kidderpore Avenue, London NW3 7ST (England), 11 May 1976.

Summary. 5-Hydroxytryptamine creatinine sulphate loses its biological activity when maintained at room temperature. The loss of 5-HT activity (in stimulating sodium transport across frog skin) is greater than the loss of creatinine sulphate activity (inhibition of sodium transport).

5-Hydroxytryptamine stimulates ion transport in a variety of tissues e.g. blood platelets[1], the nervous system at central and peripheral synapses[2], vertebrate nephrons[3], insect Malpighian tubules[4], and insect salivary glands[5] but inhibits transport in other tissues e.g. erythrocytes[6] and the nervous system[2]. 5-Hydroxytryptamine creatinine sulphate complex (5-HTCS) has been shown to have a biphasic effect on active sodium transport across isolated frog skin[7]. At low concentrations (3×10^{-5} M) transepithelial sodium transport is stimulated whereas at higher concentrations transport is inhibited. The stimulatory and inhibitory actions of 5-HTCS can be attributed to the separate components of the complex: 5-hydroxytryptamine being stimulatory and creatinine sulphate inhibitory. During the initial study of the dose-response characteristics of the induced changes in sodium transport major discrepancies in the magnitude of response became apparent in that smaller responses were consistently observed in experiments performed some hours after preparation of the 5-HTCS solution than in experiments performed using freshly prepared 5-HTCS. As the experimental protocol remained standard this suggested that the 5-HTCS solution may be losing activity so a series of experiments were designed to examine the relative activity of 5-HTCS – both solid and in solution – under a variety of conditions.

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