permanent tonic activity and rapidly propagated bursts has already been observed in the proximal colon of the rabbit[4]. On the other hand, the slow migration of strong phases of continuous activity superimposed on a low level activity as seen in cattle, seems to be an alternative pattern for the aboral transfer of the large amounts of residual food found in herbivores, since such a pattern has never been recorded in dogs[5], pigs[6] or humans[7].

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May K^+ ions stimulate the formation of cyclic AMP in the brain independently on their depolarizing action?

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Summary. The effect of potassium ions on the formation of adenosine 3',5'-monophosphate (cAMP) in the rat cerebral cortex in vivo was studied under conditions where development of spreading depression had been blocked by pretreatment of the cerebral cortex by topically applied magnesium ions. A linear relationship between potassium concentrations applied to the cortical surface and levels of cAMP has been found. Moreover, potentiation of the K^+-effect by magnesium ions has been observed.

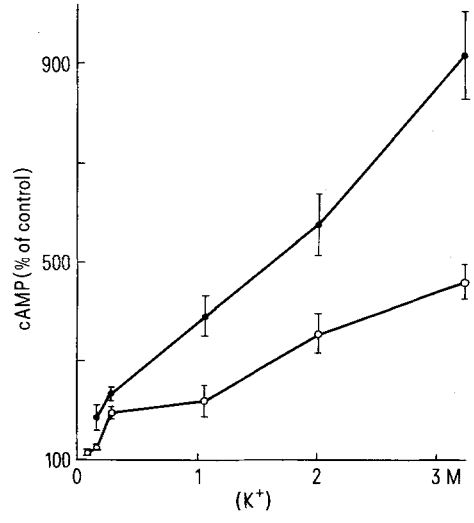
In a previous study, we have shown that a massive depolarization of cerebral cortex cells in vivo, manifested as spreading depression (SD)[1], is accompanied by an elevation of cAMP. Potassium ions, in contrast to the other depolarizing agents used, were able to stimulate the formation of cAMP, both at subthreshold concentrations for evoking SD, and at high concentrations where all the cells are completely depolarized[2-4]. This feature suggested that K^+ affects the cAMP generation system in a direct way as well as indirectly through its effect on depolarization. To test this hypothesis, the effect of K^+ on cAMP levels in the brain cortex was studied under conditions where K^+-evoked SD had been prevented by pretreatment of the cerebral cortex by topically applied magnesium ions.

Materials and methods. Male hooded rats (Druckrey strain) of 180-200 g b.wt were used throughout the experiments. Under pentobarbital anaesthesia (40 mg per kg, i.p.), trephine openings 3 mm in diameter were made in the frontal and the parieto-occipital areas. The dura was kept intact. SD was elicited by application of a filter paper (2×2 mm) soaked in KCl solutions of various concentrations into the trephine opening above the frontal cortex of one hemisphere. The contralateral frontal cortex was treated with saline in a similar way. Development of SD under the influence of K^+ was checked by the appearance of a slow potential change in the parieto-occipital cortex of the same hemisphere.

To prevent elicitation of SD by K^+, the frontal cortex was pretreated with $MgCl_2$ solutions. A concentration of 1.5 M Mg^{2+} sufficed to prevent K^+-induced SD even after concentrations of 3.2 M KCl. 2 types of experiments have been undertaken. In the first one, both frontal hemicortices were treated by topical application of a filter paper soaked with 1.5 M $MgCl_2$. After 30 min, the paper was removed, the cortical surface washed with saline and KCl applied to one of the trephine openings above the frontal hemicortex as mentioned above. The results were compared with the effects of KCl alone. In the 2nd type of experiment, the frontal cortex of only one hemisphere was pretreated with 1.5 M $MgCl_2$. After 30 min, 2.1 M KCl was applied on both frontal hemicortices. Thus the effect of Mg^{2+} on K^+-induced elevation of cAMP was studied in the same animal.

When SD did not appear in the parieto-occipital hemicortex 10 min after K^+ application, the rat was killed by immersion in liquid nitrogen. Samples of about 1 mg wet weight of frozen cortex were dissected at −20 °C and homogenized in 6% trichloracetic acid at 4 °C. cAMP was determined by a protein binding assay procedure as described earlier[3]. Protein was measured by the method of Lowry et al.[5].

Results and discussion. The figure demonstrates the effect of K^+ on cAMP levels in the brain cortex with and without Mg^{2+}-pretreatment. It appears that, in spite of the blocking of K^+-induced SD by Mg^{2+}, the stimulation of cAMP formation by K^+ in a dose-dependent manner proceeds as in the absence of Mg^{2+}, or similarly. Moreover, a remarkable potentiation of the K^+-effect by Mg^{2+} apparently occurs. This facilitation effect is rather striking and as yet unexplained. It could be due to permeability changes induced by the high $MgCl_2$-concentrations. Mg^{2+} alone did not influence cAMP levels significantly. cAMP content in



Cyclic AMP level in the brain cortex as a function of concentration of KCl solutions topically applied on to the surface of one hemicortex. Surface of both control and K^+-affected hemicortices pretreated with 1.5 M $MgCl_2$ (●—●). Surface of both hemicortices pretreated with saline (○—○).

the saline-treated cortex was 1.09 ± 0.08 pmoles/100 mg protein, whereas in the Mg-pretreated cortex, $1.25 + 0.09$ pmoles/100 mg protein was detected. To demonstrate the Mg^{2+}-facilitation effect in the same animal, an additional series of experiments was undertaken in which 2.1 M K^+-induced cAMP elevation in both normal and Mg-treated hemicortices were performed in the same animal. The following values have been obtained: 4.88 ± 0.67 and 9.03 ± 0.53 pmoles/100 mg protein for saline and Mg^{2+}-pretreated hemicortices respectively.

According to current hypotheses, depolarizing agents, including K^+, act on the cAMP-generating system indirectly, i.e. through adenylate cyclase-linked receptors activated by adenosine and glutamate released from the cells by depolarization[6-8]. Our results demonstrate, however, that potassium ions are able to stimulate cAMP formation independently of their ability to evoke SD, i.e. to induce a massive depolarization of the cortical cells. This might suggest that K^+ stimulates synthesis of cAMP in a direct way, besides that mediated by depolarization. The apparent facilitation effect of magnesium ions on the K^+-induced elevation of cAMP is difficult to explain as yet and remains to be elucidated by further experiments.

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Occurrence of inhibitory histamine H_2-receptors in isolated pulmonary blood vessels of dogs and rats

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Summary. Isolated, helically cut strips of pulmonary arteries and veins of dogs, and pulmonary arteries of rats, precontracted with norepinephrine or 5-HT exhibited potent concentration-dependent relaxations to impromidine and dimaprit (histamine H_2-agonists). The occurrence of inhibitory histamine H_2-receptors in the pulmonary vasculature could play a modulatory role in hypoxic pulmonary vasoconstriction.

It has been firmly established that histamine acts on at least 2 subtypes of receptors, namely H_1 and H_2-receptors, which may act in opposite or similar directions in the cardiovascular, respiratory and gastro-intestinal systems[2]. A dual histamine receptor population (H_1: vasoconstriction; H_2: vasodilatation) is thought to exist in the intact pulmonary circulation of guinea-pigs, dogs and cats[3]. Although a modulatory role for histamine H_2-receptors in hypoxic pulmonary vasoconstriction (HPV) has been hypothesized, at least in dogs, cats and rats[4], the exact anatomical site(s) of these histamine H_2-receptors in the pulmonary vasculature is not as yet known. In this report, we now demonstrate a potent relaxant effect of impromidine and dimaprit (2 highly specific and potent H_2-receptor agonists)[5], on isolated pulmonary blood vessels of rats and dogs.

Material and methods. Helically cut strips of pulmonary arteries and veins were prepared after sacrifice[6] from 10 adult mongrel dogs (12–22 kg), of either sex, and 15 male Wistar rats (300–450 g). The tissues were set up isometrically in isolated tissue baths containing Krebs-Ringer bicarbonate solution[6,7]. The tissues were aerated with a 5% CO_2–95% O_2 mixture, at 37 °C, under a resting load of 2 g for canine pulmonary arterial and venous strips ($2–3 \times 25–30$ mm) and 1 g for rat pulmonary arterial strips (1.5×20 mm). After 2 h of equilibration, the tissues were partially contracted with $ED_{40} \pm 10\%$ concentrations of norepinephrine bitartrate (NE) (50–100 ng/ml) or serotonin creatinine sulfate (5-HT: 10–20 ng/ml) in the case of the canine vessels; for the rat pulmonary artery 0.1–1.0 µg/ml of NE and 1–5 µg/ml of 5-HT were utilized. Responses to single or cumulative doses of impromidine and dimaprit were then recorded in duplicate. After washing and restoration of baseline tension, the agonists were then tested in the presence of the H_2-receptor antagonist, metiamide. Grass FT.03C force transducers and 4-channel Model 5 or 7 polygraphs were utilized, as described previously[7]. Where appropriate, means ± SEMs were calculated and compared for statistical significance by Student's t-test.

Results. The representative data shown in figures 1 and 2, as well as in the table, clearly demonstrate the inhibitory effects of dimaprit and impromidine on isolated pulmonary arteries and veins of dogs ($n = 10$) and rat pulmonary arteries. In general, impromidine is about 100 times more potent as a relaxant han dimaprit when ED_{50} concentrations are compared (table). In addition, impromidine produced a greater maximal relaxation than dimaprit (table).

Other experiments indicated (8 rats and 5 dogs) that metiamide (5×10^{-6} M), a specific H_2-receptor antagonist[2], failed to alter contractile responses to NE or 5-HT. However, metiamide competitively antagonized relaxant responses of both dimaprit and impromidine (e.g., figure 2; dose ratio = 2–5).

Discussion. Histamine is contained in relatively large concentrations in the lungs of rats and dogs as well as of other mammals. Histamine has been considered as a mediator, as well as a modulator of HPV[4], i.e., its exact role in HPV is still not known. Histamine is well-known to induce H_1-receptor-mediated pulmonary vasoconstriction in several mammalian species so far investigated[2]. However, under the conditions of increased pulmonary vascular tone, such as in HPV, histamine may induce pulmonary vasodilatation. Rat pulmonary arteries are relatively insensitive to the contractile action to histamine[6], but, as shown here, exhibit potent, dose-dependent relaxations to H_2-agonists. Thus, there appears to be a preponderance of H_2-inhibitory receptors in rat pulmonary arteries. On the other hand, canine pulmonary blood vessels possess an abundance of H_1-receptors, which are susceptible to blockade by