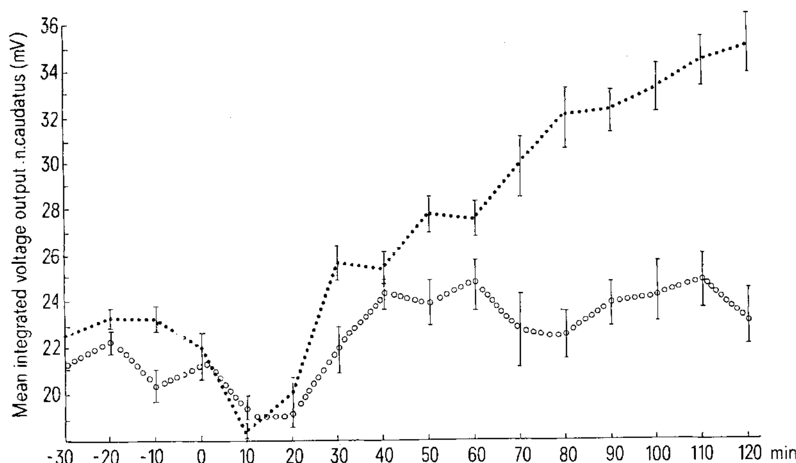

Fig. 2. Effect of IVT vehicle (○○○○) or calcitonin (8 U/kg) injection (....) on conscious rabbit brain electrogenesis at various times after administration (Mean values and S.D. of 8 animals). The integrated EEG recorded from NC is taken as representative of the tracings from all the areas considered.

data would suggest that the effect might be dose-related, but they are not included in Figure 1 because only a few animals have been so far tested.

The calcium levels after IVT calcitonin showed a progressive decrease from the basal mean value of 11.8 ± 1.1 mg/100 ml to 10.6 ± 1.3, 7.7 ± 0.5, 7.5 ± 0.2 mg/100 ml at 30, 60 and 90 min respectively. This fact indicates a leaking of the polypeptide from the brain to the peripheral blood. The significance of changes of calcium ions in analgesia is highlighted also by the reported increase of the analgesic action of morphine and other opiates consequent to a decalcifying agent administration[6]. On the other hand, an involvement of changes of calcium within the neurons induced by calcitonin seems a worthwhile hypothesis, since an influence of the polypeptide on infracellular calcium distribution is suggested as the mechanism through which calcitonin carries out its hormonal action on target cells[7].

As to the results of the EEG studies (Figure 2), we have seen an initial decrease in the average electrogenesis recorded from all the different brain areas which persists about 10 min after the IVT administration both of the vehicle and of calcitonin solution. Then, only in calcitonin-treated rabbits, a phase of progressive increase of EEG voltage follows which reaches its peak approximately in coincidence with the maximal antinociceptive effect (Figures 1 and 2). The integrated voltage values corresponding to the area of the nucleus caudatus plotted in Figure 1 are representative also of other brain areas recorded.

Considering that all the structures from which EEG tracings have been recorded showed similar changes, it is impossible to indicate a specific centre for the action of calcitonin. The parallel increase of all electrical brain potentials and of the thresholds to painful stimuli favours the possibility of a diffuse involvement of neurons' populations by calcitonin, but does not exclude that other areas, not yet investigated, may be more specifically responsive.

Under IVT administration of calcitonin, the conscious rabbits were at first behaviourally excited with signs of hypertonia particularly evident in the neck muscles. Subsequently they showed periodically restlessness with spontaneous running movements.

In conclusion, calcitonin injected into the brain ventricle elicits clear-cut effects including analgesia, the mechanism of which remains largely unknown but deserves further investigation.

Riassunto. La iniezione di calcitonina nei ventricoli cerebrali di conigli non anestetizzati induce analgesia e variazioni elettroencefalografiche con prevalente innalzamento dei potenziali elettrici di diverse aree cerebrali considerate.

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Potentiation of the Effect of Histamine by PGE_2 in the Isolated Perfused Rabbit Kidney and Guinea-Pig Lung

We recently indicated that histamine produces a vasoconstrictor effect on the isolated perfused rabbit kidney and guinea-pig lung acting on histamine H_1-receptors. The vasoconstrictor action of the amine was converted into a vasodilator one by histamine H_1-receptor blockers. The vasodilator action of histamine is due to the stimulation of H_2-receptor since the competitive inhibitors of these receptors (burimamide and metiamide) can significantly inhibit this effect[1,2]. The H_2-receptor blockers added to the perfusion medium cause a potentiation on the vasoconstrictor effect of the amine because of the elimination of masked vasodilator action

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of histamine[1,2]. In the present study we observed similar potentiation of the effect of histamine by prostaglandin E_2 (PGE_2) in the same isolated perfused organs.

Material and method. The experiments were carried out in the isolated perfused rabbit kidney and guinea-pig lung from adult animals of both sexes. After sodium pentobarbital anesthesia (30 mg/kg i.v.), the animals were injected with sodium heparin (500 U/kg i.v.), then the kidney was isolated and perfused as described previously[3]. The lungs were isolated according to the method of BAKHLE et al.[4]. Both organs were perfused with oxygenated (95% O_2 and 5% CO_2 mixture) and warmed (37 °C) Krebs' solution. The flow rate was 17 ml/min for the kidney and 10 ml/min for the lung. It was kept constant throughout the experiment. Perfusion pressure was measured by a pressure transducer (Statham p 23 Dc) and recorded on a Beckman type RB Dynograph. Urine drops were recorded simultaneously by a magnetic tipper from the cannulated ureter. The results were statistically evaluated using Student's *t*-test.

Results. Histamine caused a dose-dependent increase in both perfusion pressure and urine volume when given through the renal artery as single injections. Figure 1 shows the dose-response curves of histamine before and after addition of PGE_2 into the perfusion fluid. A parallel shift to the left was observed in the curves for each parameter after PGE_2. The estimated ED_{50} value of histamine was found to be about 4 µg/ml in control experiments and about 1 µg/ml in presence of PGE_2. Addition of metiamide (1 µg/ml) into the perfusion fluid did not cause further potentiation. Similar results were obtained in the isolated perfused guinea-pig lung as summarized in Figure 2. PGE_2 did not cause a potentiation of the effect of noradrenaline, angiotensin and serotonin.

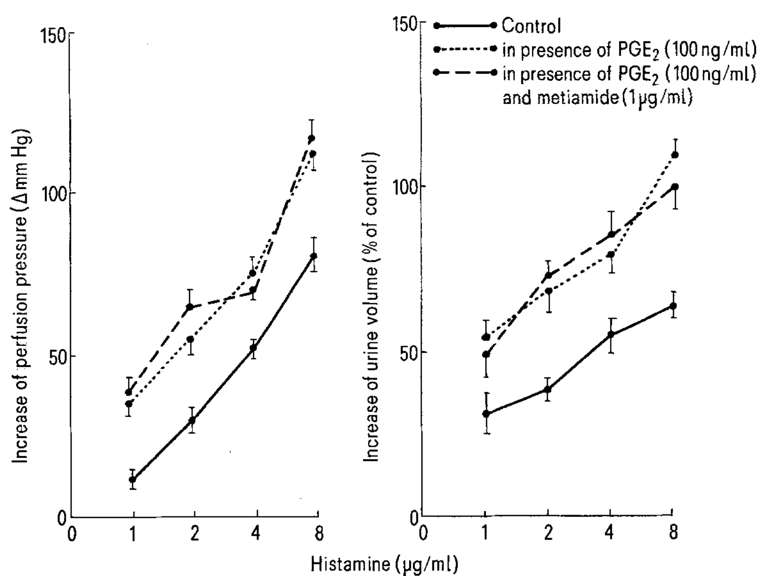

Fig. 1. The dose-response curve of histamine in the isolated perfused rabbit kidney. Each point represents the mean value of 15 experiments. Vertical bars shows S.E.M.

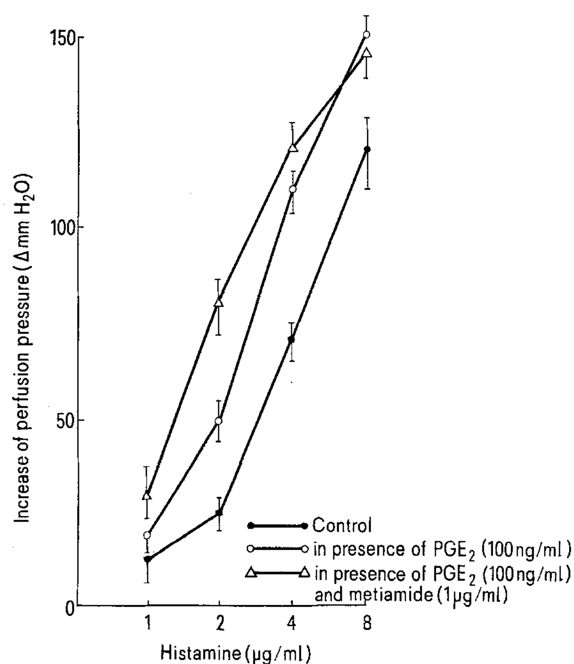

Fig. 2. The dose-response curve of histamine in the isolated perfused guinea-pig lung. Each point represents the mean value of 15 experiments. Vertical bars indicates S.E.M.

Addition of theophylline (1×10^{-5} *M*) into the fluid perfusing kidney and lung caused a significant decrease in the pressor response induced by histamine without changing the effect of noradrenaline, angiotensin and 5-HT. Theophylline also antagonized the potentiation of the pressor effect of histamine induced by PGE_2.

Discussion. The results of the present study show that PGE_2 specifically potentiates the effect of histamine but not that of noradrenaline, angiotensin II and 5-HT in the isolated perfused rabbit kidney and guinea-pig lung. We previously observed that histamine has a vasodilator effect through the stimulation of H_2-receptors in both organs[1,2]. In normal Krebs-perfused organs, this effect is masked by the pressor action of histamine which occurs by the stimulation of H_1-receptors. The blockade of H_2-receptors by burimamide can cause a potentiation of the pressor because of the elimination of masked action of the amine in opposite direction[1]. In the present study

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another H_2-receptor blocker, metiamide[5], as well as PGE_2 can induce equal potentiation in both organs. This suggests a common mechanism for both compounds. The first explanation is that PGE_2 may have a blocking effect on H_2-receptors. This seems unlikely since PGE_2 does not inhibit the relaxation (unpublished observation) induced by histamine in the cat tracheal muscle, which has been shown to be mediated by H_2-receptors[6].

It has previously been shown that histamine may stimulate the gastric acid secretion[7] and heart muscle[8] simultaneously with an increase of cellular cyclic AMP level. Both effects have been found to be blocked by burimamide. It seems likely that the stimulation of H_2-receptors in both perfused organs can produce the vasodilator effect by the increase of second messenger system, cyclic AMP. This speculation has been based upon findings obtained with theophylline, which has been described as a potent inhibitor of phosphodiesterase[9]. Addition of theophylline to the perfusion medium causes an inhibition of the pressor effect of histamine in both organs. This effect is probably due to the accumulation of cyclic AMP which represents the stimulation of H_2-receptors. Since theophylline antagonizes the potentiating action of PGE_2 on histamine responses in both organs, it is highly possible that PGE_2 and theophylline influence phosphodiesterase in opposite directions. Another possibility should be taken into consideration that PGE_2 may inhibit adenyl cyclase activity and consequently cause an inhibition of cyclic AMP in the cellular level. This point is still under investigation.

Zusammenfassung. PGE_2 verstärkt an isoliert perfundierten Kaninchennieren und Meerschweinchenlungen die Wirkung von Histamin auf den Perfusionsdruck. Gleiches wurde auch mit Metiamid, einem H_2-Rezeptorblocker, beobachtet. Anhand dieser Befunde wird die mögliche Rolle von c-AMP bei dieser Verstärkerwirkung diskutiert.

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The Metabolism of Phenylethylamine and *O*-Methylated Derivatives by Monoamine Oxidase

Phenylethylamine (PEA) and paramethoxyphenylethylamine (PMPEA) are sympathomimetic agents similar in structure to the catecholamines. Both compounds cross the blood-brain barrier and can cause central effects. PEA induces a strong, amphetamine-like central stimulation[1], while PMPEA administration results in a short-lasting catatonic state[2]. Similar catatonic responses have also been observed following the administration of 3,4-dimethyoxyphenylethylamine (3,4 DMPEA)[3]. Pretreatment with monoamine oxidase (MAO) inhibitors potentiates the depletion of central monoamines by PEA[4] and prolonges the effects of PMPEA on monosynaptic spinal reflexes[5]. Thus it is likely that PEA, and PMPEA, are metabolized in vivo by MAO, the enzyme responsible for the intraneuronal inactivation of biogenic amines. In this study we have compared the effects of PEA and PMPEA, as well as those of the dimethoxy derivatives (2,3 and 3,4 DMPEA) on brain MAO activity in vitro.

Methods of procedure. The metabolism of phenylethylamine and its derivatives was studied indirectly, by measurement of MAO activity in vitro in the presence of these agents. A water homogenate of rat brain stem (pons and medulla) was the enzyme source for these in vitro studies. MAO was measured by a modification of the micro method of MCCAMAN[6], using C^{14} tyramine as substrate. For kinetic analysis, enzyme activities were measured at several substrate concentrations in the presence of varying concentrations of the compound tested. MAO activity was expressed as millimoles of substrate deaminated/g protein/h. Data were plotted by the method of LINEWEAVER-BURK[7] to give values for K_m and V_{max}. Competitive inhibitions is observed where K_m, but not V_{max}, is changed as compared to controls. In cases of competitive inhibition, the concentration of the inhibitor necessary to produce half maximal inhibition (K_i) was calculated from the slope of the LINEWEAVER-BURK curves. For non-competitive inhibition approximate K_i values have been calculated.

Results. The interactions of PEA and PMPEA with rat brain MAO are described in Figures 1A and 1B, respectively. Although both compounds inhibit brain MAO with tyramine as substrate, the nature of the inhibition differs. PEA is a non-competitive inhibitor (Figure 1A), whereas PMPEA inhibits in a competitive manner (Figure 1B). Qualitatively and quantitatively similar results were obtained with serotonin and dopamine as substrates. The concentration of PMPEA required to give half-maximal inhibition (K_i) is 18.0 μM. For PEA a series of K_i values are obtained, in the range of 87–142 μM.

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