injections. However, although significance was not reached from the 3rd to the 5th h, the convulsive score was higher for methysergide-treated mice compared to control mice receiving CSF prior to an injection of d-amphetamine.

The figure illustrates the peak intensification effect of methysergide on amphetamine-induced convulsions elicited by handling in mice. After 2 h following drug administration, mice pretreated with methysergide (10 µg/animal i.c.) plus amphetamine (50 µg/animal i.c.) had a mean seizure score of 1.0 ± 0.15. These results indicate that methysergide intensified the convulsive response of amphetamine by 63%. These findings provide evidence for an inhibitory role of 5HT in the amphetamine-induced convulsive response.

Discussion. The findings of this study indicate that interruption of serotonergic receptor activity by utilization of methysergide, a serotonergic receptor blocker, results in an enhanced convulsive response induced by d-amphetamine in mice.

It is important to point out that amphetamine has a direct action on serotonergic receptors in a variety of smooth muscles[14,15]. In addition, our findings are in complete agreement with previous experiments reporting enhancement of amphetamine action after interruption of ascending serotonergic pathways[16].

As previously discussed, there has been considerable controversy concerning the mechanisms of amphetamine action on behavior. Along these lines, Havlicek[2] suggested that possibly CA's released by amphetamine may be part of a feedback mechanism that inhibits excessive excitation induced by the direct action of amphetamine. The possibility exists that DA released by amphetamine enters serotonergic neurons[11] and release 5HT which may serve as the inhibitory modulator.

There is considerable evidence that 5HT exerts an inhibitory effect on a variety of behaviors. The depletion of 5HT by lesions or drugs leads to an enhanced wakefulness[17], enhanced lever pressing for intracranial stimulation[18], and enhanced responsiveness to painful stimuli[19]. An alternative explanation is based on the possibility that amphetamine exerts its primary action on the catecholaminergic system. If we assume this to be the primary mechanism of amphetamine-induced excitation, then the released CA's are normally under the inhibitory control of the serotonergic system. Thus, the depletion of 5HT by p-chlorphenylalanine[20], Medial Forebrain Bundle (MFB) lesions or serotonergic receptor blockade (obtained in these studies), reduces the inhibitory influence and allows amphetamine to exert a stronger effect on behavior.

Nevertheless, our experiments further suggests that a full understanding of the behavioral effects of amphetamine must take into account the role of the serotonergic system in the central action of amphetamine.

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Cyclic nucleotide levels in the perfused rat heart subjected to hypoxia

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Summary. Isolated rat hearts were subjected to hypoxic perfusion on a recirculating Langendorff apparatus. Following a 30-min-period of aerobic stabilization the hearts were perfused for 30 min with media equilibrated with 84% N_2, 12% O_2 and 4% CO_2. At the end of the hypoxic period myocardial concentrations of cyclic AMP and cyclic GMP were determined by radioimmunoassay. Exposure to hypoxia resulted in a significant increase in cyclic AMP ($p < 0.01$) and a decrease in cyclic GMP ($p < 0.05$) as compared to hearts perfused for 60 min with media gassed with 96% O_2, 4% CO_2.

The conversion of a stimulus such as work overload, ischemia or hypoxia to a biochemical signal initiating the increase in RNA and protein synthesis observed in cardiac hypertrophy is not clear. One of the earliest events noted in models of hypertrophy such as pressure overload[3], and cardiomyopathy[4] is an increase in adenylate cyclase activity. The isolated perfused rat heart preparation subjected to hypoxia has been used in this laboratory in series of studies aimed at clarifying the possible sequence of events leading to cardiac hypertrophy. We have demonstrated that exposure of the perfused heart to 30 min of hypoxia results in a 60–100% increase in myocardial RNA synthesis following reoxygenation[5]. The purpose of the present study was to determine whether cyclic nucleotide levels are altered in hearts subjected to 30 min of hypoxia, preceding the increase in RNA synthesis observed in this model.

Materials and methods. Hearts from 230–250 g male Wistar rats (Charles River Laboratories) were perfused on a modified recirculating Langendorff apparatus as

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described in detail elsewhere[6,7]. Following 30 min of aerobic perfusion (equilibrated with 96% O_2 and 4% CO_2) the hearts were perfused for 30 min with hypoxic perfusion (12% O_2, 84% N_2, 4% CO_2). Control hearts were perfused aerobically for 60 min. Left ventricular pressure (LVP), perfusion pressure, ECG and LVdp/dt were monitored continuously on a direct-writing recorder (Beckman Dynograph type R 411).

At the end of the experimental period, hearts ventricles were freeze-clamped with aluminium tongs cooled in liquid nitrogen and pulverized under liquid nitrogen. The tissue was homogenized in 6% trichloroacetic acid and approximately 300,000 DPM of 8-^{14}C-cyclic AMP (Schwarz/Mann, 49.2 mCi/mmole) and 500,000 DPM 8-^{3}H-cyclic GMP (Amersham Searle, 13 Ci/mmole), were added as internal standards. The cyclic nucleotide were separated by passing through a AG 1-X8 column and cyclic AMP and cyclic GMP concentration were measured by radioimmunoassay[8]. Less than 1% cross contamination was observed in either cyclic AMP or cyclic GMP fractions obtained from the column. The antibody preparations were routinely checked for cross reactivity with up to 20 nmoles per tube of cyclic AMP, cyclic GMP, AMP and adenosine (Sigma Chemical Co.). Final concentrations of cyclic nucleotides determine by radioimmunoassay were corrected for recovery of ^{3}H-cyclic GMP and ^{14}C-cyclic AMP. Statistical analysis was carried out at the 5% level of significance or less by means of two-way analysis of variance with interaction.

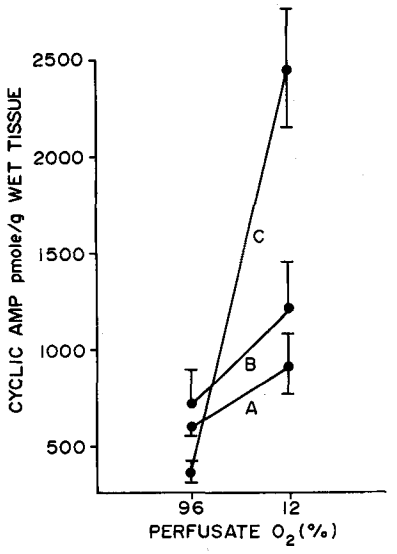

Fig. 1. The effect of hypoxic perfusion on myocardial concentration of cyclic AMP.

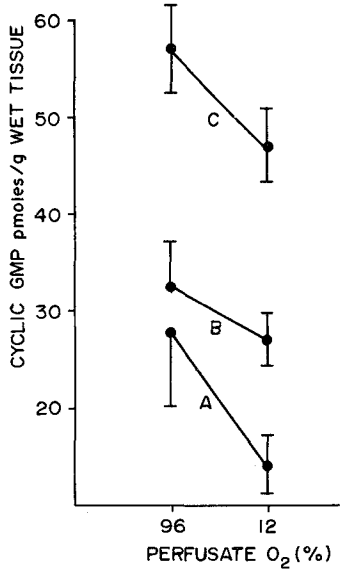

Fig. 2. The effect of hypoxic perfusion on myocardial concentration of cyclic GMP.
Each of groups A, B or C represent the concentrations of cyclic nucleotides determined in 3–4 aerobic and 3–4 hypoxic hearts using the same radioimmunoassay kit (± S. E. M.).

Results. Studies on cardiac performance, metabolic changes as well as tissue integrety following exposure to intermittent hypoxia were previously reported[6,7]. Exposure of the isolated perfused hearts to 30 min of hypoxia resulted in a significant increase in cyclic AMP (figure 1) and a significant decrease in cyclic GMP (figure 2) concentrations. The hearts were assayed in groups of 3–4 aerobic and 3–4 hypoxic preparations per radioimmunoassay kit (groups A, B and C). The means of the hypoxic groups had cyclic AMP concentrations higher by 50, 65 and 540% than those of their aerobic controls ($p < 0.01$) while cyclic GMP concentrations were lower by 18, 16 and 48% respectively ($p < 0.05$).

Considerable variation in the levels of cyclic nucleotides were observed between the different groups of hearts assayed. This may be due to differences in basal levels of the nucleotides in the different groups, or due to different degrees of specificity of the individual radioimmunoassay kits.

Discussion. Cyclic AMP has been implicated in genetic activation in prokaryotes[9] and in the stimulation of RNA synthesis in mammalian liver[10,11]. Our recent demonstration that rat hearts subjected to hypoxic perfusion exhibited increased post-hypoxic RNA synthesis[5] led us to examine the effects of hypoxia on myocardial cyclic nucleotide levels. The significant increase in myocardial content of cyclic AMP, reported in the present communication, prior to the increase in hypoxia-induced RNA synthesis suggests a possible relationship between the 2 events. Support for this kind of corrolation is suggested from altered cyclic AMP concentration seen in other models of initiation of cardiac hypertrophy. Limas and co-workers[12] reported increased levels of cyclic AMP in the rat heart 5 min after acute aortic constriction. Elevated adenylate cyclase activity was found in the hearts of myopathic hamsters[4], another model of cardiac hypertrophy. Schreiber et al.[3] demonstrated increased activity of adenylate cyclase in the particulate fraction of perfused guinea-pig hearts within 10 min of onset of pressure overload and prior to increased RNA polymerase activity.

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The significance of an increase in myocardial cyclic AMP in response to hypoxia is presently unresolved. It has been repeatedly suggested that cyclic AMP is the intracellular agent mediating the inotropic action of adrenergic amines. However, in the hypoxic state cardiac muscle is largely depleted of its high energy intermediates[5,6,13,14] and, hence, would be unable to respond to an increase in cyclic AMP content with increased contractility. The role of cyclic AMP in the conversion of glycogen phosphorylase from the b- to the more active a-form has been also well-documented. Therefore, an increase in cyclic AMP levels in the hypoxic heart might be interpreted as an attempt by the myocardium to overcome the energy depletion due to oxygen deprivation by activation of glycogen phosphorylase. Meerson[15] has suggested that the common condition preceding the initiation of cardiac hypertrophy by various causes, including work overload and hypoxia, is a relative energy deficit. He has further suggested that cyclic AMP might provide the biochemical link between energy deficit and increased RNA and protein synthesis. However, the mechanism whereby energy depletion leads to alterations in cyclic nucleotide levels has not been established.

The inherent difficulty of directly correlating increased cyclic AMP content with increased RNA synthetic activity in the perfused heart led us to investigate the effect of cyclic nucleotides on RNA synthesis in isolated myocardial nuclei. We have observed that cyclic AMP significantly stimulates the activity of RNA polymerase under conditions of low ionic strength in the presence of magnesium ion in this system (manuscript in press).

Information regarding levels of cyclic GMP in cardiac tissue has been limited to the reciprocal relationship between cyclic AMP and cyclic GMP during the contraction cycle of frog hearts beating at low rates[16], and to the demonstration of an increase in cyclic GMP associated with a decrease in contractility in the isolated perfused heart following administration of acetylcholine[17,18]. The significance of a decrease in myocardial cyclic GMP in response to hypoxia noted in this study requires additional investigation.

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Cerebral application of enkephalins

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Summary. Implantation of enkephalins A or B into the ventral thalamus or injection into the lateral ventricle of rats evoked only weak signs of stereotyped behavior, but did not cause gnawing.

Since the discovery and structure-determination of enkephalins[1,2], these pentapeptides have become available by synthesis[3]. The high biological activity of these peptides is usually demonstrated in vitro by their morphine-like activity on the guinea-pig ileum or on the mouse vas deferens[1,4]. In vivo applications are, however, handicapped by the fact that the peptides undergo rapid enzymatic hydrolysis[2].

We have shown recently that deposition of small amounts of morphine (50–100 µg on each side) into the ventral thalamus evokes stereotyped behavior in rats[5]. This is a stimulatory effect and is easily recognisable. Still smaller amounts of the alkaloid are effective if injected into the lateral ventricle of the rat[6]. Since in such topical applications the chances of metabolic survival are greater – e.g. the CSF is known to have a very low level of proteins and of enzymic activities – we have tried both implantation of 20 or 50 µg into the ventral thalamus and intraventricular injection of 10 or 50 µg, using (methionin-)enkephalin A as well as (leucine-)enkephalin B. On the basis of in vitro experiments[1,2], these amounts should be equivalent to the same or larger doses of morphine. For each dosage, 10 male rats of 150–200 g body weight were used. For implantation into the thalamus, aqueous solutions of the peptides were mixed with talc and the material was dried at room temperature. For detailed description of the technique, see reference 5.

In all animals which received the higher doses, and in some with the lower amounts of enkephalins, we found signs of central excitation such as rubbing and licking, but in no case did the full picture of stereotypy develop, i.e. the stage of biting and gnawing was not reached. 3 h after application, all rats were quiet and somewhat depressed.

Thus even with these forms of topical application, the full effect of enkephalins on morphine receptors has not been unequivocally demonstrated. Apparently the survival of extrinsic enkephalins in cerebral tissue is not sufficiently prolonged to permit the long-lasting stimulation which is required by the animals to develop morphine-induced gnawing. The latter appears within 1–3 h after topical application of the alkaloid. Perhaps better results could be obtained with continuous infusion of enkephalins into the ventricles; however the material available was insufficient for this purpose.

Another possibility must also be considered, viz. that in the brain larger peptides, like the C-fragment of Bradbury et al.[7] are required to activate the opiate receptors efficiently.

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