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CONTROL OF PROSTAGLANDIN BIOSYNTHESIS IN RAT BRAIN HOMOGENATES BY ADENINE NUCLEOTIDES

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Abstract—The control of prostaglandin biosynthesis by adenine nucleotides was studied in rat brain homogenates. ADP stimulated PGE_1 Formation. Cyclic AMP enhanced PGE_2 biosynthesis. The formation of PGE_3 was inhibited by ATP. The action of some drugs on the control system was studied. The results are used for a hypothesis on the mechanism of depressive illness.

IT HAS been proposed that the lesion in depressive illness may be diminished formation of cyclic 3′, 5′ AMP in the brain and other tissues.[1] It was also postulated that in mania the cyclic nucleotide may be formed in excess. The hypothesis explained some of the clinical features and laboratory findings in affective disorders. Certain important characteristics of the disease, however, were not accounted for. These include the diurnal swing of mood in depression, the self-limiting nature of the attacks, the alternation of mania and depression in some cases and the intracellular sodium retention in may patients.[2] The mechanism of electroconvulsive therapy and lithium prophylaxis remained enigmatic. In this report a scheme for the mutual control of adenine nucleotide levels and prostaglandin formation is outlined. It is used as a basis for a hypothesis of affective illness in which the disease is seen to be a lesion of the control system rather than a defective metabolic pathway.

METHODS

All experiments were done on rat brain homogenates. Eight animals were used. All the tests were carried on each brain. A whole brain was ground in 10 ml of 50 mM Tris–HCl containing 1 mM EDTA, using a tightly fitting all glass hand homogenizer. The suspension was centrifuged at 700 *g* for 15 min. The supernatant was used as a source of prostaglandin synthesising activity. Electrical stimulation of the homogenate was achieved by passing through the supernatant 5 square pulses each 120 V and of 0·03 msec duration.

Prostaglandin synthesising activity was assayed by adding 0·05 ml of brain homogenate to 1 ml of a solution containing 1 mM of the appropriate 1-C^{14}-labelled precursor fatty acid (50,000 count/min) as well as 0·3 mM reduced glutathione, 0·3 mM hydroquinone, 20 mM Tris–buffer pH 7·4, 130 mM KCl and 10 mM $MgCl_2$. After incubation for one hour at 37°, the reaction was stopped by adding 1 ml of 0·2 M citric acid. Prostaglandins were extracted with two aliquots of ether. The ether extract was resolved on silica thin layer chromatoplates developed in benzene–dioxane–acetic acid (20:20:1 v/v/v).[3] Areas corresponding to a marker prostaglandin E_1 were scraped for radioactivity measurement by liquid scintillation counting.

Drugs were added to the incubation mixture at the following concentrations; amitryptyline 10^{-6} M, haloperidol 10^{-8} M and chlorpromazine 10^{-7} M. 1-C^{14}-Labelled eicosatrienoic acid, arachidonic acid and eicosapentaenoic acid were isolated from *Euglena gracilis* Z grown in the dark on a synthetic medium containing 1-C^{14}-linoleic acid.[4] Adenyl cyclase was determined by the method of Krishna.[5]

RESULTS AND DISCUSSION

Nucleotide prostaglandin interactions. Prostaglandin E_1 stimulates the formation of cyclic AMP.[6] Prostaglandin E_2 (PGE_2) stimulates adenylate kinase and the formation of ADP.[7] In the presence of PGE_3, ADP inhibits adenylate kinase,[7] and adenyl

TABLE 1. THE EFFECTS OF PGE_1, PGE_3 AND CYCLIC AMP ON ADENYL CYCLASE ACTIVITY IN RAT BRAIN HOMOGENATES AND IN LYSED HUMAN BLOOD PLATELETS

Enzyme source	Additions				
	None	1 μM PGE_1	1 μM PGE_3	1 μM PGE_3 10 μM cyclic AMP	10 μM cyclic AMP
Rat brain homogenate	11 ± 9%	20 ± 8%	10·5 ± 7%	6·1 ± 6%	11·1 ± 6%
Lysed human blood platelets	9 ± 8%	20 ± 6%	9·2 ± 7%	4·2 ± 8%	9·3 ± 7%

Enzyme activity is expressed as μ-moles of cyclic AMP formed per hr per milligram protein. Each result represents the mean of six experiments.

cyclase is inhibited by cyclic AMP (Table 1). Thus PGE_3 prevents the accumulation of ADP and cyclic AMP.

ADP enhances the formation of PGE_1 (Fig. 1). PGE_2 synthesis is augmented by cyclic AMP (Fig. 2). ATP[1] inhibits the formation of PGE_3 (Fig. 3). The action of

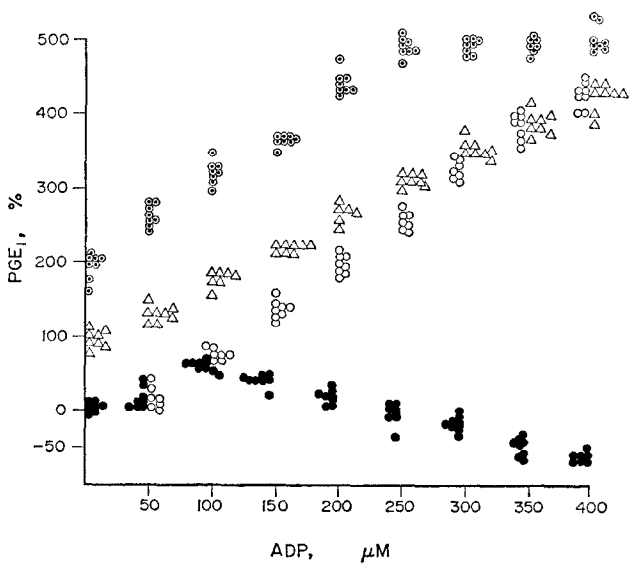

FIG. 1. The effect of ADP on PGE_1 synthesis (○) and its modification by amitryptyline (△), Na (●) and electricpulse (⊙). Changes are expressed as percentage of PGE_1 formed in the absence of nucleotides and modifiers (data from eight brains).

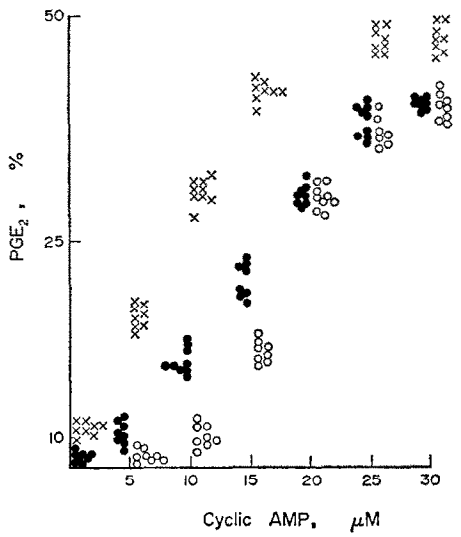

FIG. 2. The effect of cyclic AMP on PGE_2 formation (⊙) and its modification by lithium (●) and haloperidol (×). Changes are expressed as percentage of PGE_2 formed in the absence of nucleotides and modifiers (data from eight brains).

each nucleotide on the biosynthesis of the corresponding prostaglandin is concentration dependent and the function follows a sigmoidal curve. The nucleotide prostaglandin interactions are summarized in Fig. 4.

Action of ions, drugs and electrical stimulation. In the presence of Na (20 mM) cyclic AMP stimulates PGE_1 synthesis (Fig. 5). The enhancement is proportional to the cyclic nucleotide concentration up to 15 μM. Cyclic AMP levels higher than 15

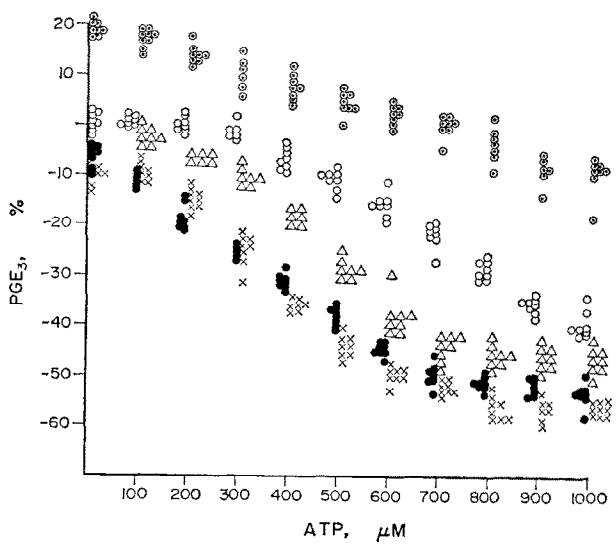

FIG. 3. The effect of ATP on PGE_3 formation (△) and its modification by Na (×), lithium (○), amitryptyline (●), and haloperidol (⊙). Changes are expressed as percentage of PGE_3 formed in the absence of nucleotides and modifiers (data from eight brains).

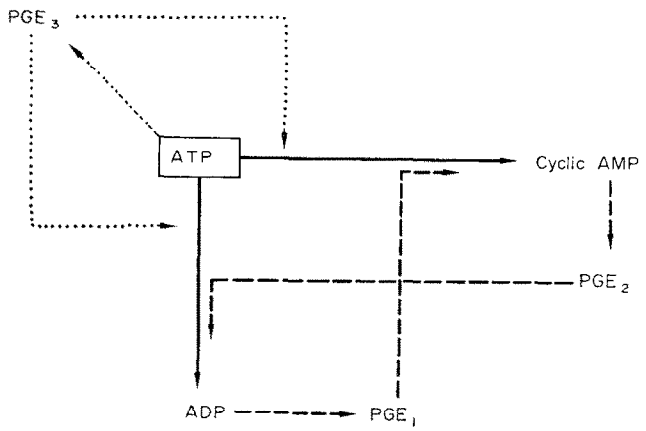

FIG. 4. The diagram shows: (i) The intracellular formation of cyclic AMP and ADP from ATP; (ii) The effect of these nucleotides on the formation of prostaglandins E_1, E_2 and E_3; (iii) the control of nucleotide pathways by prostaglandins, ——— Conversion, – – – – Stimulation, ... Inhibition.

μM progressively inhibit PGE_1 biosynthesis. Sodium ions also cause PGE_1 formation to be inhibited by ADP (Fig. 1). The inhibition is proportional to ADP concentration. PGE_3 formation is inhibited by Na ions (Fig. 3).

Lithium mildly augments PGE_3 synthesis. It diminishes the sigmoidicity of the curve for cyclic AMP–PGE_2 formation (Fig. 2).

Amitryptyline stimulates PGE_1 synthesis (Fig. 1) and abolishes the sigmoidicity of the ADP–PGE_1 curve. It also inhibits the formation of PGE_3 (Fig. 3).

The passage of electrical pulses in rat brain homogenate causes a very marked enhancement of PGE_1 formation (Fig. 1). The synthesis of PGE_3 is mildly stimulated.

Haloperidol stimulates PGE_2 and PGE_3 biosynthesis. It renders PGE_2 formation more sensitive to cyclic AMP concentration (Fig. 2). On the other hand, it causes ATP inhibition of PGE_3 production to be more gradual and regular (Fig. 3).

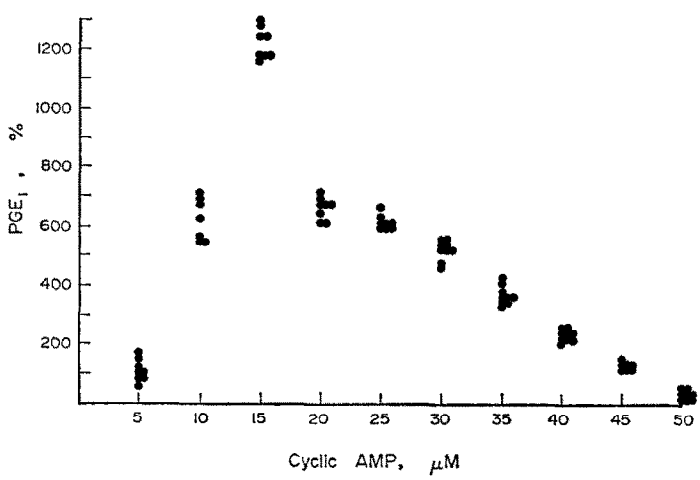

FIG. 5. The effect of cyclic AMP on the biosynthesis of PGE_1 in presence of Na. There is a stimulation at low concentrations and inhibition at high concentrations of the nucleotide. In absence of Na, cyclic AMP has no control on PGE_1 formation.

Chlorpromazine abolishes sigmoidicity in all curves of nucleotide-prostaglandin formation. The interrelationships described in the preceding paragraphs were derived from experiments with rat brain homogenates containing all subcellular components. The action of nucleotides, cations and drugs on prostaglandin synthesis may not be due to the direct influence on prostaglandin synthetase enzymes. It is possible that some of the factors may affect prostaglandin synthesis by the mediation of one or more steps.

Pathogenesis of affective disorders. It is hazardous to extrapolate from experiments with rat brain homogenates to the psychiatric pathology of human patients. However, the highly speculative scheme suggested in the following paragraphs could be a basis for further research. Its embrasiveness was motivated by a desire for inner consistency, and should not be considered as an evidence for its validity.

Increase in intracellular Na may precipitate manic depressive disease by the following mechanism. In the presence of Na^+, cyclic AMP (up to 15 μM) stimulates the formation of PGE_1 and hence more cyclic AMP (Fig. 5). This means that, instead of a corrective control, there is an explosive feed back mechanism for increased formation of cyclic AMP. Na^+ by diminishing the formation of PGE_3 increases the limit of allowable cyclic AMP. Thus the cyclic AMP concentration may rise to a level that can produce mania. A high concentration of cyclic AMP has a corrective feed back inhibition of PGE_1 formation (Fig. 5). It also stimulates the formation of PGE_2 leading to a high level of ADP. In presence of 20 μM sodium, ADP inhibits PGE_1 synthesis (Fig. 1). This may cause over correction of the PGE_1 level, bringing cyclic AMP to the level found in depression. Thus, the self perpetuating process can start another cycle.

A plausible mechanism for stress depression, is that, under such conditions, ATP breakdown may be increased. Fall in the ATP level may relieve the suppression of PGE_3 formation, which inhibits adenyl cyclase.

Recurrent depression could arise if the ADP–PGE_1 curve is more sigmoidal than normal. A small drop, within the normal range, of ADP concentration would lead to a significant drop in PGE_1 formation leading to a pathologically low level of cyclic AMP (Fig. 6). Such a condition would also lead to diurnal swing of mood. A steep sigmoid curve accounts for precipitation of depression; it also implies that PGE_1 formation (hence cyclic AMP synthesis) is more sensitive to ADP increase. ADP formed from ATP breakdown during the activities of the day would lead to a sharper increase in PGE_1 and cyclic AMP than it would under normal conditions. Hence the increase of cyclic AMP in evening leads to improvement of mood.

If the cyclic AMP—PGE_2 is markedly sigmoidal this could lead to recurrent mania. With a slight drop in cyclic AMP, there would be a marked decrease in PGE_2 and ADP levels below the normal value (Fig. 6). This may be corrected by increased inhibition of PGE_3 formation by increase in ATP. This correction would lead to an "overshoot" increase in cyclic AMP.

Lithium may protect against recurrence of mania by its inhibitory effect on PGE_3 production and by stimulating PGE_2 formation at low ADP concentrations.

Electrical stimulation enhanced PGE_1 synthesis (Fig. 1). It is conceivable that this may be one of the mechanisms by which electroconvulsive therapy causes remission in depression. Amitryptyline has a similar, though less marked action. It also abolishes the lag phase in the ADP–PGE_1 formation curve.

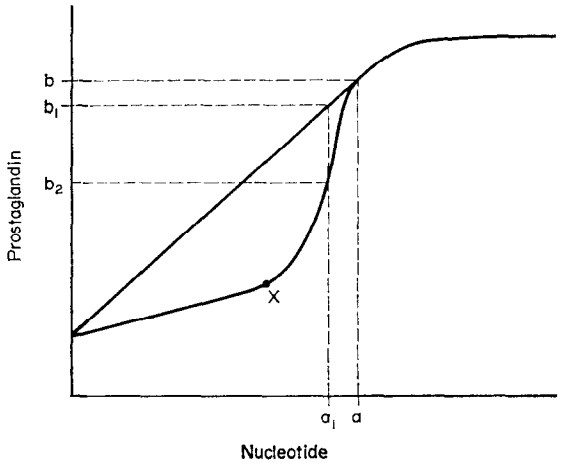

FIG. 6. Illustrates the effect of small changes in a nucleotide on the formation of the prostaglandin stimulated by the nucleotide. If the relationship follows hyperbolic curve, a small drop in the nucleotide level (from a to a_1) leads to a small decrease in prostaglandin formation (from b to b_1). However, if the interaction follows a sigmoid curve, such a small decrease in the nucleotide level would lead to a marked fall in prostaglandin formation (from b to b_2). In a sigmoid function prostaglandin formation is relatively insensitive to changes in nucleotide concentrations below X, and markedly sensitive to nucleotide change above that level.

The efficiency of haloperidol in the treatment of mania could be due to the fact that it stimulates the biosynthesis of PGE_2, directly as well as by potentiating the stimulating action of cyclic AMP (Fig. 2). The drug also maintains a high level of PGE_3 which checks the activity of adenyl cyclase (Fig. 3).

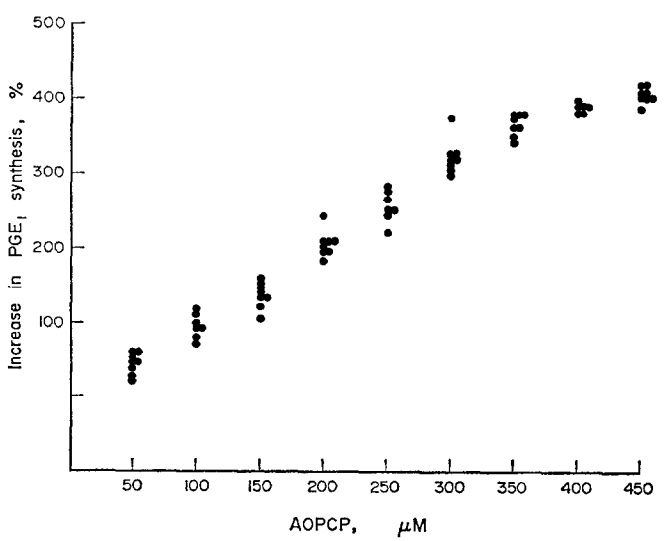

FIG. 7. The stimulation of PGE_1 formation by α, β methylene adenosine diphosphate. Increments are expressed as percentage of PGE_1 produced in the absence of the nucleotide.

The phosphonic acid analogue of ADP* stimulates the synthesis of PGE_1 (Fig. 7) and is metabolically inert.[8] It may have potential antidepressive activity. We found that intraperitoneal injection of 0·5 mg of the analogue into 30 g mice 15 min before reserpine (1 mg/kg body weight) inhibited the development of ptosis to an extent comparable to 2 mg of imipramine. The action is dose dependent in the range 0·1–1 mg. Injection of the drug 24 hr after reserpinization abolished motor retardation and restored exploratory and grooming behaviours. However, there was no detectable change in blepharospasm. The phosphonic nucleotide (0·5 mg) was far superior to imipramine in relieving catalepsy, diarrhoea and hypothermia of chronic reserpinization (1 mg reserpine/kilogram body weight/day for 1 week).

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* α-β-Methylene adenosine diphosphate supplied by Miles Laboratories Inc.