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Effects of Prostaglandins on Cyclic AMP Content of Superior Cervical Ganglia of the Cow and Cat[1]

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Dopamine and prostaglandin E_2 (PGE_2) produced time- and concentration-dependent increases in cyclic AMP levels of bovine superior cervical ganglia (SCG). Maximal increases in cyclic AMP levels were produced within 1 min by PGE_2, compared to 10 min for dopamine. Dopamine and PGE_2 were synergistic in their effects on cyclic AMP levels. By contrast, prostaglandin $F_{2\alpha}$ had no effect on cyclic AMP levels. In the feline SCG, the modest increases in cyclic AMP levels produced by dopamine were not statistically significant. Neither PGE_2 nor $PGF_{2\alpha}$ increased cyclic AMP levels in feline SCG. Interpretation: Dopamine increased cyclic AMP synthesis in bovine SCG by stimulating a dopamine receptor-adenylate cyclase complex. PGE_2 increased cyclic AMP synthesis in bovine SCG by stimulating an adenylate cyclase complex. This complex may or may not be the same one stimulated by dopamine. The stimulatory effect of PGE_2 is specific for prostaglandins of the E series, since $PGF_{2\alpha}$ had no effect. Neither dopamine nor PGE_2 stimulated cyclic AMP synthesis in the feline SCG because this ganglion lacks significant adenylate cyclase activity.

INDEX DESCRIPTORS: Cyclic AMP, Prostaglandins, Superior Cervical Ganglion, Synaptic Transmission.

Cyclic AMP (cAMP) is a key intermediate in neural transmission in the superior cervical ganglion (SCG). Preganglionic stimulation not only activates the principal ganglionic neurons (PGNs), but also the small intensely fluorescent (SIF) cells. The stimulated SIF cell releases dopamine which binds to a dopamine receptor-adenylate cyclase complex (ACC) on the postsynaptic membrane of the SIF cell-PGN synapse. Activation of the ACC causes cAMP synthesis, hyperpolarizing the PGN by the generation of a slow inhibitory postsynaptic potential (s-IPSP) (Greengard, 1975, 1976, 1978; Greengard and Kebabian, 1974; Libet, 1970, 1977; McAfee et al., 1971).

Dopamine causes elevation of the cAMP content of bovine (Kebabian and Greengard, 1971) and rabbit SCG (Wamsley et al., 1980). It also hyperpolarizes rabbit SCG (McAfee and Greengard, 1972). The rabbit SCG exhibits a s-IPSP (Libet, 1970); however this potential is weak or absent in cat SCG (Haefely, 1974). Thus, the rabbit and cow SCG offer an opportunity to test for responses which are absent in the cat SCG (Black et al., 1978). Prostaglandins of the E series are known to elevate cyclic AMP levels in a wide variety of tissues, whereas those of the F series do not (Brody and Kadowitz, 1974; Coceani, 1974). Therefore it seemed important to determine the effect of dopamine, PGE_2 and $PGF_{2\alpha}$ on the cyclic AMP content of cat and cow SCG.

MATERIALS AND METHODS

Bovine SCG were dissected at the abattoir within 5 min of death by exsanguination. They were placed in ice-cold 0.32 M sucrose for transport to the laboratory, where they were trimmed of fat and connective tissue and diced into cubes approximately 1 mm on a side. Bovine SCG were pre-incubated in vitro in a Dubnoff shaking incubator at 37°C for 20 min in Eagle's Minimum Essential Medium containing 5 mM theophylline. The incubation medium was then made 50 μM with respect to dopamine, and the incubation continued. At the termination of the experiment, tissues were frozen and ground in liquid nitrogen using a porcelain mortar and pestle. They were then homogenized using a Polytron tissue disintegrator (Brinkmann Inst. Co., Westbury, NY) at 4°C in 6% (w/v) trichloroacetic acid (TCA) containing 800 d.p.m. tritiated cAMP as an internal standard. The homogenates were centrifuged at 13,000 × g for 15 min at 4°C in a Sorvall RC-2B superspeed centrifuge (Sorvall Inc., Newtown, CT). The precipitate was dissolved in 1.0 N NaOH and analyzed for protein by the method of Lowry et al. (1951). The supernatants were extracted 6 times with an equal volume of diethyl ether saturated with double-distilled water. Residual amounts of ether were removed by placing samples in a water bath at 40°C for a few minutes until the odor of ether disappeared. The samples were then frozen, lyophilized, and assayed for cAMP by the method of Gilman (1970, 1972; Gilman and Murad, 1974).

After determination of the time course for dopamine stimulation of cAMP synthesis, the concentration-dependency of the stimulation of cAMP synthesis by dopamine was determined. Bovine SCG were pre-incubated as before, then incubated with various concentrations of dopamine for 10 min at 37°C. Tissues were frozen in liquid nitrogen and processed as before.

Prostaglandin E_2 elevates cAMP levels in a wide variety of tissues whereas $PGF_{2\alpha}$ does not. The time course for the stimulation of cAMP synthesis by PGE_2 was determined. Bovine SCG were pre-incubated as before, then incubated in 50 μM PGE_2 at 37°C for various lengths of time, frozen and analyzed for cAMP. Since maximal cAMP synthesis occurred within 1 min, the concentration-dependency was determined using various concentrations of PGE_2 incubated for 1 min at 37°C. The effect of $PGF_{2\alpha}$ on cAMP synthesis was determined by pre-incubating bovine SCG as before, then incubating them in 50 μM $PGF_{2\alpha}$ for 1 min at 37°C.

Cat SCG were isolated surgically under Nembutal anesthesia (35 mg/kg body weight, i.p.). Ganglia were removed, desheathed, and pre-incubated in Eagle's Medium containing 5 mM theophylline for 20 min at 37°C. They were then incubated at 37°C in either 50 μM dopamine for 10 min or in various concentrations of the prostaglandins for 1 min. After incubation SCG were homogenized and analyzed for cAMP.

The possibility that dopamine and PGE_2 might act together to produce greater increases in cAMP levels than the sum of their individual effects on cAMP generation was tested by pre-incubating ganglia as before, then incubating them in 50 μM dopamine for 9 min. The incubation medium was then made 50 μM with respect to PGE_2, the incubation continued for 1 min, and ganglia frozen in liquid nitrogen and analyzed for cAMP.

RESULTS

The increases in cAMP levels produced by dopamine were time-dependent. Note that dopamine-stimulated cAMP production became constant after 10 min (Table 1). A similar time course has been published by Roch and Kalix (1975). The stimulation of cAMP levels by dopamine was concentration-dependent (Table 2). PGE_2 also in-

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Table 1. *Effects of Dopamine and PGE_2 on Cyclic AMP Levels in Bovine SCG.*

Incubation Time (Min)	Cyclic AMP (picomoles/mg protein) ± S. E. Dopamine[a]	PGE_2[b]
0	28.8 ± 2.2 (6) (100%)	28.8 ± 2.2 (6) (100%)
1		46.4 ± 3.3 (7) (161%)
3	100 ± 15 (6) (347%)[c]	44.5 ± 6.2 (7) (155%)
5	125 ± 12 (6) (434%)[c]	41.3 ± 4.4 (7) (143%)
10	149 ± 15 (6) (517%)[c]	38.0 ± 1.4 (4) (132%)
15	156 ± 15 (6) (542%)[c]	38.1 ± 4.7 (8) (133%)
17.5	151 ± 21 (6) (524%)[c]	26.2 ± 3.2 (8) (91.0%)
20	155 ± 17 (6) (538%)[c]	21.3 ± 1.5 (7) (74.0%)

[a]Cyclic AMP levels produced by pre-incubating bovine SCG with 5 mM theophylline for 20 min at 37°C, then incubating in 50 μM dopamine for various lengths of time. The number of samples is in the 1st parenthesis, and the percentage of control values in the 2nd parenthesis.

[b]Cyclic AMP levels produced by pre-incubating bovine SCG with 5 mM theophylline for 20 min at 37°C, then incubating in 50 μM PGE_2 for various lengths of time. The number of samples is in the 1st parenthesis, and the percentage of control values in the 2nd parenthesis.

[c]The increases produced were statistically significant ($P<0.01$, Student's "t" test).

Table 2. *Concentration-Dependency of Cyclic AMP Increases*

Agonist	Concentration[a] μM	Cyclic AMP (picomoles/mg protein)[b] ± S. E. Bovine SCG	Feline SCG
Dopamine	100	156 ± 15 (5) (541%)[d]	43.1 ± 7.8 (6) (160%)
Dopamine	50	151 ± 21 (6) (524%)[d]	41.0 ± 4.5 (4) (152%)
Dopamine	10	118 ± 12 (5) (410%)[d]	41.1 ± 5.9 (5) (153%)
Dopamine	5	102 ± 10 (5) (353%)[d]	46.7 ± 12 (4) (174%)
Dopamine	1	66.3 ± 16 (5) (230%)[d]	35.0 ± 4.0 (4) (125%)
PGE_2	100	58.1 ± 4.0 (5) (202%)[e]	26.5 ± 2.1 (4) (99%)
PGE_2	50	46.4 ± 3.3 (7) (161%)[e]	22.2 ± 1.5 (6) (82%)
PGE_2	10	30.8 ± 2.9 (6) (107%)	25.0 ± 1.5 (6) (93%)
$PGF_{2\alpha}$	50	29.9 ± 3.0 (5) (104%)	26.0 ± 2.5 (6) (97%)
Control		28.8 ± 2.2 (6) (100%)	26.9 ± 2.7 (6) (100%)
50 μM DA + 100 μM PGE_2[c]		305 ± 15 (6) (1059%)[d]	41.2 ± 3.7 (6) (153%)

[a]Micromolar concentration of agonist in incubation medium. Samples were incubated for 10 min (dopamine) or for 1 min (PGE_2, $PGF_2\alpha$).

[b]Number of samples is in the 1st parenthesis, and percentage of control values in the 2nd parenthesis.

[c]Cyclic AMP content after incubating SCG in 50 μM for 10 min, and making the medium 100 μM with respect to PGE_2 during the last min of incubation. Note the synergistic effect of dopamine and PGE_2 on cyclic AMP levels in bovine SCG.

[d]The increases produced were statistically significant ($P<0.01$, Student's "t" test).

[e]The increases produced were statistically significant ($P<0.05$, Student's "t" test).

creased cAMP levels in bovine SCG in a time-dependent manner (Table 1). Note that the time required to produce maximal amounts of cAMP after PGE_2 stimulation was approximately 1 min, compared to 10 min for dopamine. cAMP levels began to decline after a plateau was reached, dropping below control values some 17.5 min later (Table 1). $PGF_{2\alpha}$ did not increase cAMP levels in bovine SCG. Dopamine appeared to produce modest increases in cAMP levels in the cat SCG, but they were not statistically significant by Student's "t" test ($P>0.05$) (Table 2). Neither PGE_2 nor $PGF_{2\alpha}$ elevated cAMP levels in cat SCG. Dopamine and PGE_2 were synergistic in their effects on cAMP production in bovine SCG, as the increases produced by stimulation with both compounds were considerably greater than the sum of the increases produced by stimulation with either compound alone (Table 2).

DISCUSSION

Dopamine stimulates cAMP synthesis in bovine SCG, the increases becoming time-independent after 10 min. PGE_2 also increases cAMP levels, but the increases become time-independent after 1 min. This time difference may reflect the time required for the charged dopamine molecule to migrate to the postsynaptic membrane of the SIF cell-PGN synapse. The lesser time required for PGE_2 reflects the lipophilic nature of this compound, permitting easier migration through cellular membranes. Note that cAMP levels generated by 50 μM PGE_2 are not as high as those produced by 50 μM dopamine. Prostaglandins of the E series usually stimulate adenylate cyclase activity, while those of the F series do not. $PGF_{2\alpha}$ was without effect on cAMP levels of either bovine or feline SCG.

Dopamine is thought to stimulate the dopamine receptor of a dopamine receptor-adenylate cyclase complex (ACC) in bovine SCG (Greengard and Kebabian, 1974; Greengard, 1975, 1976, 1978). PGE_2 is thought to exert its effect by direct stimulation of adenylate cyclase activity. The synergistic results obtained with combined stimulation by dopamine and PGE_2 are consistent with this concept. 50 μM dopamine exerts a maximal effect on the dopamine receptor. If PGE_2 were to stimulate the dopamine receptor, no synergy between dopamine and PGE_2 should be observed. However, dopamine stimulation of the dopamine receptor and PGE_2 stimulation of adenylate cyclase activity could result in synergy upon combined stimulation with both compounds (providing the rate of adenylate cyclase activity were not rate-limiting). PGE_2 might also stimulate adenylate cyclase activity not associated with the dopamine receptor-ACC.

The increases in cAMP produced by PGE_2 in bovine SCG began to decline almost immediately, dropping below control values within 20 min. We suggest that this decrease is due to the induction of phosphodiesterase activity. Schwartz and Passonneau (1974) showed that increases in cAMP levels cause induction of phosphodiesterase activity in the C-6 rat glioma cell line. Phosphodiesterase induction occurred whether dibutyryl cAMP was added directly to the medium or intracellular cAMP levels were increased indirectly by stimulation of a β-adrenergic receptor-ACC with isoproterenol. Increases were noted at 30 min, the earliest time tested. Gilman and Nirenberg (1971) produced maximal increases in cAMP levels by stimulating rat C-6 glioma cell cultures with 100 μM isoproterenol. Decreases in the maximal levels observed began 15-20 min after the beginning of stimulation. These results obtained in different neural tissues suggest that the elevation of cAMP levels triggers a mechanism which decreases cAMP levels. In view of the work of Schwartz and Passonneau (1974), induction of phosphodiesterase activity may be responsible for the decrease in cAMP levels observed in these experiments.

In contrast to bovine SCG, the feline SCG does not exhibit a statistically significant response to stimulation by dopamine. PGE_2 is thought to stimulate the adenylate cyclase moiety of a receptor-ACC. PGE_2 failed to stimulate cAMP production in feline SCG, indicating the absence of a receptor-ACC. These results may be interpreted as follows: 1) PGE_2 stimulates cAMP synthesis in bovine SCG by activating an ACC. The stimulatory effect is specific for prostaglandins of the E series, $PGF_{2\alpha}$ being without effect. 2) Dopamine and PGE_2 exert synergistic effects on cAMP synthesis in bovine SCG. Dopamine stimulates a dopamine receptor-ACC. The ACC stimulated by PGE_2 may be identical with the dopamine receptor-ACC, or a completely separate ACC. (Both possibilities could also be true). 3) PGE_2 does not stimulate cAMP synthesis in feline SCG because this ganglion lacks significant ACC activity. It appears that dopamine may cause a slight increase in cAMP in the feline SCG. Yet PGE_2 causes no increase whatsoever. Two explanations are possible for this apparent discrep-

ancy between dopamine and PGE_2. PGE_2 may not be as effective an agonist as dopamine (cf. results in the cow, Table 2). Alternatively, there may be a second ACC (in addition to the dopamine receptor-ACC) in cow SCG. Deficiency of such a ACC in the cat SCG would account for the absence of an effect of PGE_2 compared to the slight apparent effect of dopamine in this species.

Kalix et al. (1974) demonstrated the existence of a dopamine receptor-ACC in the rabbit SCG associated with the hyperpolarizing response and generation of a s-IPSP in PGNs. McAfee and Greengard (1972) studied the effect of different compounds on the membrane potentials of rabbit SCG after blockade of action potentials by hexamethonium or *d*-tubocurarine. According to the s-IPSP hypothesis, stimulation of cAMP synthesis in this ganglion should lead to hyperpolarization, making the PGNs more resistant to firing. In the rabbit SCG, theophylline caused membrane hyperpolarization, consistent with the ability of theophylline to increase cAMP levels by blocking phosphodiesterase activity. Dopamine and monobutyryl cAMP each caused membrane hyperpolarization, and theophylline prevented the dopamine-induced hyperpolarization. However, PGE_2 caused membrane depolarization, and reversed the dopamine-induced membrane hyperpolarization.

Such results are consistent with a decrease rather than an increase in cAMP, although prostaglandins of the E series usually increase cAMP levels. An explanation for this apparent discrepancy may be found in the results reported in Table 1. PGE_2 elevates cAMP levels initially, although they decrease below control values by 20 min. McAfee and Greengard (1972) added PGE_1 30 min before beginning their electrophysiological studies. It seems possible that PGE_1 initially increased cAMP levels, which subsequently declined below control values before the electrophysiological observations were made. Such a biphasic response would account for the *apparent* depolarization observed by McAfee and Greengard (1972). This distinction is important because the ability of PGE_1 to depolarize neurons has been cited as a reason for denying a role for cAMP in neural transmission (Busis et al., 1978). Thus, the effect of prostaglandins of the E series on the electrophysiology of the SCG needs further study in view of the possibility that these compounds may produce an initial membrane hyperpolarization by increasing cAMP levels in SCG.

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