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EVIDENCE AGAINST A PHYSIOLOGICAL ROLE OF cAMP IN CHOLERESIS IN DOGS AND RATS

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Abstract—The relationship between liver cAMP concentration and bile flow was studied after administration of several compounds which increase the bile acid independent flow (BAIF). In the dog, the three drugs tested (glucagon, aminophylline and DB cAMP*) increased bile flow whereas only two of them (glucagon and DB cAMP) increased liver cAMP concentrations. In the rat, none of the drugs tested (glucagon, aminophylline and MIX†) modified bile flow whereas two of them (glucagon and MIX) increased liver cAMP concentrations. Therefore, there were no parallel variations between the increase in liver cAMP concentrations and the increase in choleresis. Furthermore, (1) no relationship was found between the time course of accumulation of cAMP and the increase in bile flow and (2) no relationship was found between the magnitude of the cAMP accumulation and the increase in bile flow. These data do not support the view that cAMP has a physiological role in bile formation.

The mechanisms of canalicular bile formation include at least two processes: one related to bile acid secretion, the other probably related to Na^+–K^+ ATPase mediated sodium transport [1, 2]. It has been postulated that glucagon [3–5] and theophylline [5, 6] which increase the bile acid independent fraction (BAIF) in the dog might act through an increase in liver cAMP content [4, 6]. However in these studies, bile flow and cAMP changes, were not measured simultaneously. In a few studies, the possible role of cAMP on bile formation has been tested by administration of an exogenous derivative of cAMP, dibutyryl cAMP (DB cAMP). In the rat, DB cAMP did not modify bile flow [7], but in contrast, Morris [8] has shown that injection of DB cAMP in the dog promoted an increase in the BAIF. Similar results have been found in man by Levine [9]; biliary cAMP and bile flow were increased after DB cAMP administration. Because of these contradictory results, we carried out a study designed to re-examine the relationship between cAMP and bile flow in dogs and rats.

MATERIALS AND METHODS

Experimental procedure for dogs

Protocols. After an overnight fast, 13 mongrel dogs (11–26 kg) were anesthetized with 50 mg/kg of pentobarbital (Nembutal, Abbott Labs, St Rémy-sur-Avre, France) and maintained under artificial respiration. Body temperature was not monitored. After cystic duct ligation, the common bile duct was cannulated with a polyethylene catheter and bile was collected every 15 min in graduated test tubes. A sodium taurocholate solution (Maybridge Biochemical Corp., Tintagel, U.K.) was i.v. infused at a constant rate of 0.6 μmole.min^{-1} throughout the experiment. Pipenzolate methyl bromide (Piptal, Roger Bellon Labs, Neuilly-sur-Seine, France) was infused at a rate of 1.15 μmoles.min^{-1} during the first hr and 0.58 μmole.min^{-1} thereafter to minimize spontaneous variations of bile flow [10]. After a control period of 60–90 min (control bile flow being taken as the average flow of the last 30 min of the control period), the drugs (glucagon, theophylline, DB cAMP) were administered as indicated in the next paragraph. 45 min after the beginning of drug administration, flow was measured for 30 min and the average flow during this period was taken as flow during drug administration. For DB cAMP experiments, the period of equilibration after drug administration was reduced to 30 min. Three liver samples (50–100 mg each) were taken during the control period and at various intervals after drug administration for cAMP determination. In the experiments with DB cAMP, [^{14}C]erythritol biliary clearance was measured in order to estimate canalicular bile flow [11, 12]: [^{14}C]erythritol (Radiochemical Centre, Amersham, U.K.) was i.v. infused at a rate of 0.1 μCi.min^{-1} after a single injection of 5 μCi in dogs given DB cAMP. After an equilibration period of 1 hr, blood samples were taken for determination of [^{14}C]activity.

Drugs. Glucagon (Novo Industrie Pharmaceutique, Paris, France) was i.v. administered as a single dose of 50 μg followed by an infusion of 10 μg.kg body wt^{-1} h^{-1}. Aminophylline (Carena, Delagrange Lab.) was i.v. administered as a single injection of 280 μmoles followed by a constant infusion of 20 μmoles.min^{-1}. N^6-$O^{2'}$-dibutyryl adenosine 3′5′ cyclic AMP (DB cAMP) (Boehringer) was infused for 30 min at a rate of 0.4 μmole.kg body wt^{-1} min^{-1} according to previous results that indicated

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* N^6-$O^{2'}$-Dibutyryl adenosine 3′5′ cyclic AMP.

† 1-Methyl, 3-isobutylxanthine.

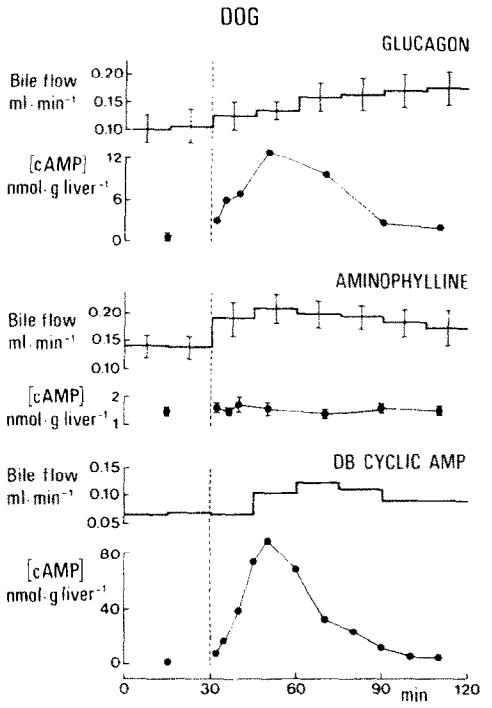

Fig. 1. Effect of glucagon, aminophylline and DB cAMP on bile flow and liver cAMP concentrations in the dog. After a control period of 60–90 min (control bile flow was taken as the two last 15 min collection periods) drugs were administered. Control cAMP concentration was the mean value of three determinations during the control period. Glucagon was administered as a single dose of 50 μg followed by an infusion of 10 $\mu g.kg^{-1}.h^{-1}$. Aminophylline was administered as a single injection of 280 μmoles followed by a constant infusion of 20 μmoles. min^{-1}. The lower panel represents a typical experiment with DB cAMP; the drug was infused for 30 min at a rate of 0.4 $\mu mole.kg^{-1}.min^{-1}$.

maximal increase in liver cAMP under these conditions [13].

Experimental procedure for rats

Protocols. Male Sprague–Dawley rats (Charles River, C.D.) fed *ad libitum* were anesthetized with 5 mg.100 g body wt^{-1} of pentobarbital (Nembutal). The temperature was maintained between 37.5 and 38.5° on a heating table. The common bile duct was cannulated and bile was collected every 10 min in weighed tubes. A sodium taurocholate solution (Maybridge) was infused at a rate of 0.2 μmole. min^{-1}.100 g body wt^{-1} throughout the experiments. After a control period of 60-min, the drugs (glucagon, aminophylline, 1-methyl,3-isobutylxanthine (MIX)) were administered as indicated in the next paragraph. Control bile flow was taken as the average flow of the last 20 min of the control period. Forty min after the beginning of glucagon and MIX administration, or 10 min after the beginning of aminophylline infusion, bile flow was measured during 20 min and the average flow during this period was taken as flow for drug administration. Four liver samples (50–100 mg) were taken during the control period and at various times after drug administration for cAMP determination. A group of six rats (200 g) was studied for each drug experiment.

Drugs. Glucagon (Novo) was infused at a rate of 10 $\mu g.100$ g body $wt^{-1}.h^{-1}$. Aminophylline (Carena) was i.v. administered as a single dose of 2.8 μmoles followed by a constant infusion of 0.27 $\mu mole.100$ g body $wt^{-1}.min^{-1}$. MIX (Aldrich) in NaCl 0.15 M ethanol (5 : 1, v/v) was i.p. injected at a dose of 4 $\mu moles.100$ g body wt^{-1}.

Analytical procedures

Liver fragments weighing about 50 mg were immediately frozen in liquid nitrogen. Tissue samples were homogenized in 2.5 ml ice-cold 10% trichloracetic acid to which had been added 2 nCi of 8[^{3}H]-cAMP (27 Ci/mole) (Radiochemical Centre, Amersham, U.K.) as tracer. The homogenates were centrifuged at 4000 rpm for 10 min at 4°. The supernatants were pipetted off, extracted six times with 10 ml diethylether after HCl was added to a final concentration of 0.1 N and the residues after ether extraction lyophilized. The residues after lyophylization were taken up in the assay buffer. The radioactivity of an aliquot was determined to allow quantitation of the recovery of cAMP through the extraction procedure. Recovery of cAMP averaged 85 per cent.

Cyclic AMP concentration was determined in duplicate according to the method of Gilman [14] except in dog experiments with DB cAMP; in the latter experiments cAMP concentrations were determined by radioimmunoassay according to the method of Steiner [15] using kits supplied by Schwarz–Mann (Orangeburg, NY).

Bile acid concentration in bile was measured by an enzymatic method using 3 α-hydroxysteroid dehydrogenase (Worthington Biochemical Corp., Freehold, NY) [16].

[^{3}H]cAMP activity and [^{14}C]erythritol activity in plasma and bile were measured after addition of 10 ml of Dimilume (Packard) in a liquid scintillation spectrometer (Intertechnique SL 40). Correction for quenching was made by external standardization.

[^{125}I]activity was measured in a scintillation spectrometer (Intertechnique CG).

Table 1. Influence of dibutyryl cAMP on bile flow, bile acid secretion and erythritol clearance in three dogs

Experiment number	Bile flow ml.min⁻¹		Bile acid secretion μmoles.min⁻¹		B/P*		Erythritol clearance ml.min⁻¹	
	Control	Drug	Control	Drug	Control	Drug	Control	Drug
1	0.067	0.116	7.52	8.87	2.37	1.89	0.158	0.220
2	0.039	0.057	7.78	7.85	3.77	3.63	0.146	0.209
3	0.080	0.143	9.37	9.83	3.14	2.29	0.250	0.330

* B/P = Bile–plasma concentration ratio of [^{14}C]erythritol.

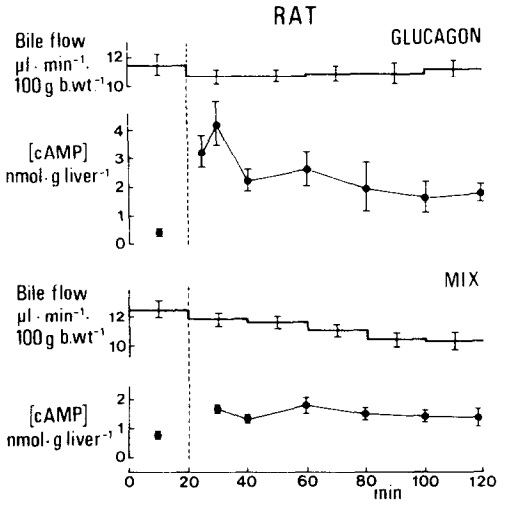

Fig. 2. Effect of glucagon and MIX on bile flow and liver cAMP concentrations in the rat. After a control period of 60-min (control bile flow was taken as the flow of the last 20 min) drugs were administered. Control cAMP concentration was the mean value of four determinations during the control period. Glucagon was infused at a rate of 10 µg. 100 g body wt^{-1}. h^{-1}. MIX was i.p. injected at a dose of 4 µmoles. 100 g body wt^{-1}. Mean values ± S.E.M. are represented.

Statistical analysis was made using the Student's '*t*' test.

RESULTS

Dog

Glucagon. The effect of glucagon on hepatic cAMP levels and bile flow is shown in Fig. 1. The time course of increase in liver cAMP was similar in all six dogs, but the magnitude of the stimulation induced by glucagon differed from one dog to another. For this reason, only mean values of cAMP levels are represented. During glucagon infusion cAMP concentrations increased rapidly: accumulation of the nucleotide was detected 2 min after glucagon administration, they reached a maximum within 10–20 min and then declined slowly despite the continued infusion of the hormone; however cAMP concentrations remained higher than basal concentrations. The mean value of cAMP concentration during the control period in the six dogs was 1280 ± S.E.M. 180 pmoles.g liver^{-1}. Maximal stimulation induced by glucagon (calculated as the ratio : maximal cAMP concentration over cAMP concentration during the control period) ranged from 3.1 to 39.2. Bile flow increased within the first 15 min, reached a maximum at approximately 30 min and remained stable thereafter. On the average bile flow increased from 0.102 ± S.E.M. 0.029 ml.min^{-1} during the control period to 0.170 ± S.E.M. 0.031 ml.min^{-1} ($P<0.01$) when maximal bile flow was attained. Bile acid secretion rate increased slightly but not significantly (8.86 ± S.E.M. 0.90–9.85 ± S.E.M. 0.46 µmole.min^{-1}).

Aminophylline. Hepatic cAMP levels did not change during aminophylline infusion in the four dogs studied (Fig. 1). None of the values taken during aminophylline administration was significantly different from the control values (1025 ± S.E.M. 154 pmoles.g liver^{-1}). Aminophylline infusion was followed by an immediate increase in bile flow, from a mean control value of 0.140 ± S.E.M. 0.016 ml.min^{-1} to 0.190 ± S.E.M. 0.023 ml.min^{-1} ($P<0.001$). Bile acid output did not change (7.96 ± S.E.M. 0.48–7.40 ± S.E.M. 1.29 µmoles.min^{-1}).

DB cAMP. A typical experiment with DB cAMP is represented in Fig. 1. The term cAMP concentration indicates cAMP concentration in the basal conditions and the sum DB cAMP plus cAMP concentrations during drug administration, since with the method used, the two compounds are measured. A striking accumulation of cAMP was observed, cAMP concentration reaching 40, 42 and 54 times the control value about 20 min after the beginning of the infusion. Bile flow increased 15 min after the beginning of drug administration, reached a maximum at approximately 30 min and then declined slowly. A similar pattern was observed in three dogs. Variations in bile flow and bile acid secretion are indicated in Table 1. Bile–plasma concentration ratio of [^{14}C]erythritol was slightly decreased while erythritol clearance increased proportionately to bile flow in the three experiments (Table 1), indicating that choleresis was of canalicular origin.

Rat

Glucagon. Glucagon administration induced a striking increase in liver cAMP concentration as seen in Fig. 2. On the average cAMP concentration increased from 439 ± S.E.M. 53 pmoles.g liver^{-1} during the control period to 4217 ± S.E.M. 817 pmoles.g liver^{-1} 10 min after the beginning of glucagon infusion; cAMP concentrations then declined but remained elevated at 4 to 6 times the control value throughout the experiment. Bile flow was not significantly modified by glucagon (11.48 ± S.E.M. 0.76–10.86 ± S.E.M. 0.71 µl.min^{-1}.100 g body wt^{-1}). Bile acid concentration declined slowly throughout the experiment and bile acid secretion was significantly decreased (0.438 ± S.E.M. 0.037–0.370 ± S.E.M. 0.021 µmoles.min^{-1}.100 g body wt^{-1}; $P<0.02$).

Aminophylline. Hepatic cAMP concentrations did not change during aminophylline infusion: on the average cAMP concentration was 656 ± S.E.M. 80 pmoles.g liver^{-1} during the control period and 750 ± S.E.M. 52 µmoles.g liver^{-1} 30 min after the beginning of aminophylline infusion. Bile flow (12.48 ± S.E.M. 0.61–11.58 ± S.E.M. 0.41 µl.min^{-1} 100 g body wt^{-1}) and bile acid secretion rate (0.381 ± S.E.M. 0.039–0.356 ± S.E.M. 0.019 µmole.min^{-1}. 100 g body wt^{-1}) did not change during aminophylline infusion.

1-Methyl, 3-isobutylxanthine. Administration of MIX increased significantly ($P<0.01$) cAMP concentration from an average control value of 860 ± S.E.M. 130 pmoles.g liver^{-1} to 1630 ± S.E.M. 99 pmoles.g liver^{-1} 10 min after MIX injection. The concentration of cAMP was stable throughout the experiment (Fig. 2). Bile flow did not change (10.18 ± S.E.M. 0.51–10.99 ± S.E.M. 0.65 µl.min^{-1}.100 g body wt^{-1}) whereas bile acid secretion rate

was significantly decreased (0.404 ± S.E.M. 0.019–0.351 ± S.E.M. 0.020 μmole.min^{-1}.100 g body wt^{-1}; $P < 0.02$).

DISCUSSION

This study was designed to test the hypothesis that cAMP might regulate the BAIF. There were no parallel variations between the increase in liver cAMP concentrations and the increase in choleresis. In the rat none of the drugs increased bile flow whereas two of them (glucagon and MIX) increased liver cAMP. In the dog, the three drugs tested increased bile flow whereas only two (glucagon and DB cAMP) increased liver cAMP content. These observations suggest therefore, that in the rat cAMP is probably not involved in the control of canalicular bile salt independent choleresis. In support of this view, it has already been reported that i.v. administration of DB cAMP did not modify bile flow [7]. At the doses used, theophylline did not modify cAMP concentration; it has already been reported that theophylline did not increase cAMP in the isolated rat liver preparation [17]; theophylline may however increase cAMP at higher doses [18].

The interpretation of the findings in the dog may be more difficult, since glucagon and DB cAMP increased both bile flow and cAMP concentration in the liver. However, aminophylline also increased bile flow without any increase in cAMP concentration. This suggests that the increase in choleresis and the effect on cAMP may be two separate effects of these drugs. This interpretation is further supported by two additional observations: (1) there was no parallelism between the time course of the increase in cAMP and of the choleretic effect and (2) there was no apparent relationship between the magnitude of the choleretic response and that of the increase in cAMP in the liver. One cannot, however, totally exclude a role of cAMP for the following reasons. Firstly, it is clear that interpretation of experiments *in vivo* is difficult because of the multiplicity of events possibly involved. Secondly, minimal or no detectable increase in cAMP have been shown to produce full activation of protein kinase [19] and to increase phosphorylase activity [20]; this could explain the choleretic effect of aminophylline without any detectable increase in cAMP. Thirdly, it is possible that a local high concentration of cAMP is responsible for the physiological events; such local concentration changes would not have been detected by the method used. Evidence for two compartments of cAMP has been presented in the toad bladder cell, one pool controlling water flow and the other sodium transport [21].

In summary, in the rat glucagon and MIX increased cAMP in the liver and did not increase bile flow; in the dog, theophylline increased bile flow but did not increase cAMP in the liver, while glucagon and DB cAMP increased both. These data do not support the view that cAMP has a physiological role in bile formation.

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REFERENCES

1. S. Erlinger and D. Dhumeaux, *Gastroenterology* **66**, 281 (1974).
2. E. L. Forker, *Ann. Rev. Physiol.* **39**, 323 (1977).
3. R. S. Jones, R. E. Geist and A. D. Hall, *Gastroenterology* **60**, 64 (1971).
4. A. Khedis, M. Dumont, M. Duval and S. Erlinger, *Biomedicine* **21**, 176 (1974).
5. J. Barnhart and B. Combes, *Proc. Soc. exp. Biol. Med.* **150**, 591 (1975).
6. S. Erlinger and M. Dumont, *Biomedicine* **19**, 27 (1973).
7. A. L. Baker and M. M. Kaplan, *Gastroenterology* **70**, 577 (1976).
8. T. Q. Morris, *Gastroenterology* (abstract) **62**, 187 (1972).
9. R. A. Levine and R. C. Hall, *Gastroenterology* **70**, 537 (1976).
10. R. Preisig, H. L. Cooper and H. O. Wheeler, *J. clin. Invest.* **41**, 1152 (1962).
11. E. L. Forker, *J. clin. Invest.* **46**, 1189 (1967).
12. H. O. Wheeler, E. D. Ross and S. E. Bradley, *Am. J. Physiol.* **214**, 866 (1968).
13. R. A. Levine, *Clin. Pharmac. Ther.* **11**, 238 (1970).
14. A. G. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
15. A. L. Steiner, C. W. Parker and D. N. Kipnis, *J. biol. Chem.* **247**, 1106 (1972).
16. P. Talalay, *Methods biochem. Analyt.* **8**, 119 (1960).
17. J. H. Exton, G. A. Robison, E. W. Sutherland and C. R. Park, *J. biol. Chem.* **240**, 20, 6166 (1971).
18. M. Costa, C. A. Manen and D. H. Russell, *Biochem. biophys. Res. Commun.* **65**, 75 (1975).
19. C. V. Byus, M. Costa, I. G. Sipes, B. B. Brodie and D. H. Russel, *Proc. natn. Acad. Sci. U.S.A.* **73**, 1241 (1976).
20. C. Ingebretsen, J. F. Clark, D. O. Allen and J. Ashmore, *Biochem. Pharmac.* **23**, 2139 (1974).
21. J. Flores, P. A. Witkum, B. Begckman and G. W. G. Sharp, *J. clin. Invest.* **56**, 256 (1975).