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MODE OF STIMULATORY ACTION OF DEOXYCHOLATE IN SIGNAL TRANSDUCTION SYSTEM OF ISOLATED RAT PANCREATIC ACINI[1]

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Mode of stimulatory action of deoxycholate (DCA) on the secretagogue-induced amylase release and the phospholipase C reaction in isolated rat pancreatic acini was investigated using sodium fluoride (NaF), which is a direct activator of GTP-binding proteins (G proteins). DCA enhanced the amylase release induced by submaximal concentrations of NaF without affecting the maximal level of this reaction. Under the similar conditions, DCA enhanced the NaF-induced phospholipase C reaction. These stimulatory effects of DCA on the NaF-induced amylase release and phospholipase C reaction are comparable to those on the secretagogue-induced reactions reported previously. These results suggest that DCA acts on the coupling of a G protein(s) to the phospholipase C in the membrane transduction mechanism in isolated rat pancreatic acini.

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Abbreviations used are: DCA, deoxycholate; CCK8, cholecystokinin-octapeptide; G protein, GTP-binding protein; NaF, sodium fluoride; CCK, cholecystokinin; Hepes, N-3-hydroxylethyl-piperazine-N'-2-ethanesulfonic acid.

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A preceding report from our laboratories (1) has reported a new action of bile acids on the exocrine pancreas different from their destructive effects described previously (2,3). This stimulatory action of bile acids on the exocrine pancreas is presumably involved in the development of biliary pancreatitis due to cholelithiasis which is a major entity in acute pancreatitis. Low concentrations of DCA , a secondary bile acid, sensitize the pancreatic acini and enhance the CCK8-induced amylase release. DCA potentiates the phospholipase C-catalyzed phosphoinositide hydrolysis which is induced by the secretagogue and thereby enhances amylase release without affecting the binding of CCK8 to its receptors on acini or secretory processes subsequent to the protein kinase C activation and the intracellular Ca^{2+} mobilization (1). It has been indicated that DCA enhances amylase release through the enhancement of the phospholipase C reaction, but the mode of stimulatory action of DCA on the phospholipase C reaction remains to be clarified.

Recently, the regulatory mechanism for the phospholipase C by receptors has been intensively investigated. Accumulating evidence suggests that a G protein(s) is involved in the coupling of receptors to the phospholipase C (4-12) by analogy with the adenylate cyclase system (13,14). Fluoride has recently been shown to be a direct activator of G proteins (15,16). It has been also shown that NaF can mimic the action of CCK in amylase release and the phospholipase C reaction in pancreatic acini (7).

In this paper, we show that DCA enhances the NaF-induced amylase release and phospholipase C reaction in isolated rat pancreatic acini. Mode of action of the stimulatory effect of DCA in the secretagogue-induced phospholipase C reaction is discussed.

MATERIALS AND METHODS

Materials and chemicals employed were purchased as described previously (1). Isolated pancreatic acini were prepared from a starved male Wistar rat (200-250 g) by the method of Williams (17). Amylase release was assayed as described previously (1). Amylase activity was determined also as described previously (1). The amylase release was expressed as the ratio of the value of the amylase activity released into the medium during the incuba-

tion to that of the total amylase content. The total amylase content was estimated by measuring the enzymatic activity of a 1-ml aliquot of the unstimulated acinar suspension after disruption of the acinar cells by sonication. Diacylglycerol and inositol phosphate formations were assayed as described previously (1). Radioactivity of ^{3}H-labeled sample was determined using a Beckman liquid scintillation system, Model LS 3801.

RESULTS

Effect of DCA on the NaF- and CCK8-induced Amylase Release

Figure 1A shows the effect of DCA on the NaF-induced amylase release. Incubation of the acini with NaF induced marked amylase release in a dose-dependent manner. The maximal and half maximal effects were observed with 10 mM and 5 mM of NaF, respectively.

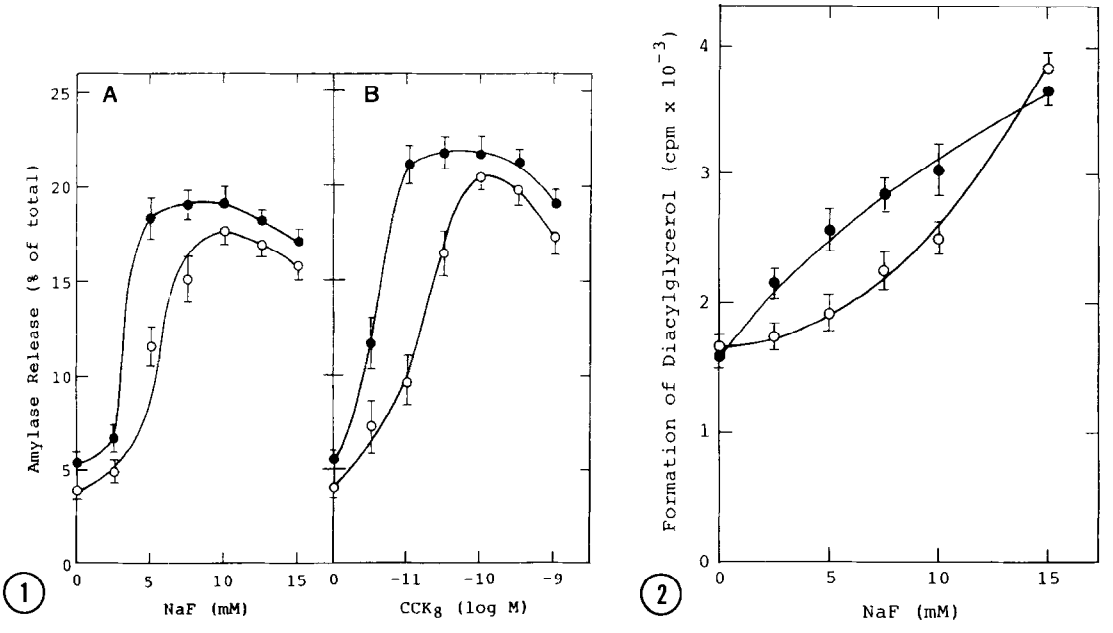

Fig. 1. Effect of DCA on the NaF- or CCK8-induced amylase release. The acini were incubated for 20 min at 37°C with various doses of NaF or CCK8 in the presence or absence of 0.25 mM DCA. (A), with various doses of NaF. (●), in the presence of DCA. (○), in the absence of DCA. (B), with various doses of CCK8. (●), in the presence of DCA. (○), in the absence of DCA. Results are the means ± SE of 3 independent experiments.

Fig. 2. Effect of DCA on the NaF-induced formation of diacylglycerol. The acini prelabeled with [^{3}H]arachidonic acid as described in "Materials and Methods" were incubated with 10 min at 37°C with various doses of NaF in the presence or absence of 0.25 mM DCA. (●), in the presence of DCA. (○), in the absence of DCA. Results are the means ± SE of 3 independent experiments.

These results are consistent with the results reported by Matozaki et al. (7). When 0.25 mM DCA was added to the acini during the incubation with NaF, the dose-response curve was shifted to left. DCA markedly enhanced amylase release induced by submaximal doses of NaF. However, DCA showed a little effect on this reaction induced by the maximal doses of NaF.

The incubation of the acini with CCK8 induced amylase release in a dose-dependent manner as shown in Fig. 1B. The maximal and half maximal effects were observed with 1×10^{-10} M and 3×10^{-11} M of CCK8, respectively. When 0.25 mM DCA was added to the acini during the incubation with CCK8, the dose-response curve was shifted to left and DCA markedly enhanced amylase release induced by submaximal doses of CCK8. However, DCA affected the maximal level of amylase release only slightly. These results are consistent with our previous results (1). The mode of stimulatory effect of DCA on the amylase release induced by NaF is basically the same as that on the amylase release induced by CCK8.

Effect of DCA on the NaF-induced Phospholipase C Reaction

Incubation of the acini with NaF caused the formation of diacylglycerol and inositol phosphates in dose-dependent manners as shown in Figs. 2 and 3, respectively. The addition of DCA to the acini during the incubation with NaF caused the enhancement of the formation of diacylglycerol and inositol phosphates induced by submaximal doses of NaF. DCA showed a little effect on these reactions induced by maximal doses of NaF. DCA alone induced neither the formation of diacylglycerol nor that of inositol phosphates in the doses used in these experiments. The effects of DCA on the NaF-induced formation of diacylglycerol and inositol phosphates were comparable to those on the CCK8-induced reactions as reported previously (1), except the effect of DCA on the formation of inositol trisphosphate. In the previous report from our laboratories (1), we described that DCA had differential actions in the formation of three species of inositol phosphates, i.e., DCA enhanced the formations of inositol monophosphate and inositol bisphosphate, but did not show a significant effect on the formation of inositol trisphosphate induced by the secreta-

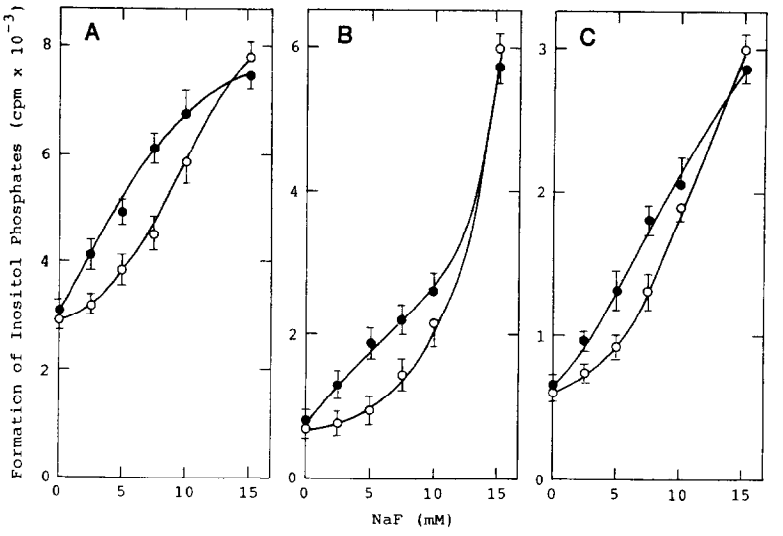

Fig. 3. Effect of DCA on the NaF-induced formation of inositol phosphates. The acini prelabeled with myo-[^{3}H]inositol as described in "Materials and Methods" were incubated for 10 min at 37°C with various doses of NaF in the presence or absence of 0.25 mM DCA. (A), inositol monophosphate; (B), inositol bisphosphate; (C), inositol trisphosphate. (●), in the presence of DCA. (○), in the absence of DCA. Results are the means ± SE of 3 independent experiments.

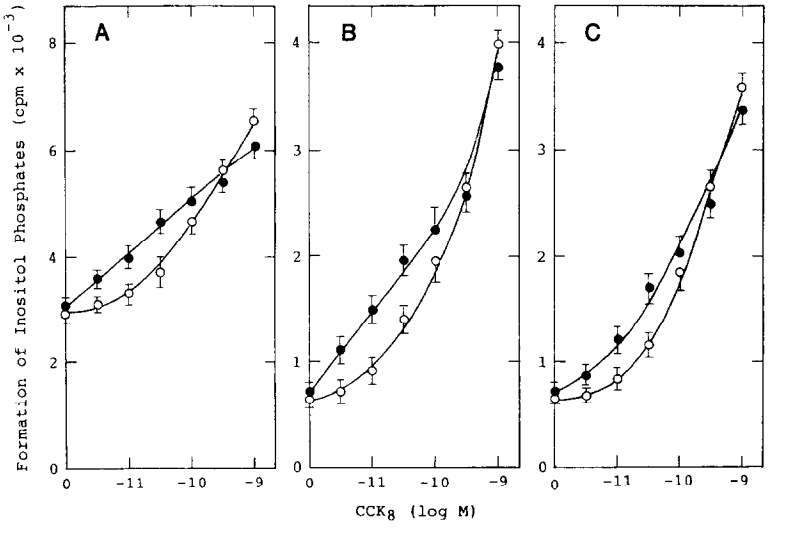

Fig. 4. Effect of DCA on the CCK8-induced formation of inositol phosphates. The acini prelabeled with myo-[^{3}H]inositol as described in "Materials and Methods" were incubated for 5 min at 37°C with various doses of CCK8 in the presence or absence of 0.25 mM DCA. (A), inositol monophosphate; (B), inositol bisphosphate; (C), inositol trisphosphate. (●), in the presence of DCA. (○), in the absence of DCA. Results are the means ± SE of 3 independent experiments.

gogue. This discrepancy was derived from the different experimental conditions; the acini prelabeled with myo-[^{3}H]-inositol were incubated for 1.5 min with the secretagogue in the previous report (1), whereas the prelabeled acini were incubated with NaF for 10 min in this paper. As shown in Fig. 4, when the prelabeled acini were incubated with CCK8 for 5 min, DCA enhanced the formation of inositol trisphosphate as well as the formation of other inositol phosphates.

DISCUSSION

Our previous report showed that DCA enhances the secretagogue-induced phospholipase C reaction and thereby potentiates amylase release. However, the mode of action of DCA on the phospholipase C reaction was unclear. Recently, it has been shown that a G protein(s) is involved in the coupling of the CCK receptor to the phospholipase C in pancreatic acinar cells (12,13). In addition, it has been also shown that fluoride, in the form of NaF, can activate the G protein(s) coupled to the phospholipase C and mimic the action of CCK in pancreatic acini (12). In the present study, we have demonstrated that DCA enhances the amylase release induced by the submaximal doses of NaF. This effect of DCA on the NaF-induced amylase release is comparable to that on the CCK8-induced amylase release. The effect of DCA on the NaF-induced phospholipase C reaction is also comparable to that on the CCK8-induced reaction. These observations together with our previous results (1) suggest that DCA acts on the processes between the coupling of the CCK8 receptor to the G protein(s) and the hydrolysis of phosphoinositides by the phospholipase C in the membrane transduction system.

There are three possible sites of action of DCA, i.e., coupling of the CCK8 receptor to the G protein(s), coupling of the G protein(s) to the phospholipase C, and the phospholipase C-catalyzed phosphoinositide hydrolysis. Among these three sites, the first one is less possible because DCA enhanced the amylase release and the phospholipase C reaction induced not only by CCK8 but also by NaF which is a direct activator of the G protein(s). It is possible that DCA acts on the coupling of the

fluoride-sensitive G protein(s) to the phospholipase C and enhances the phospholipase C activity resulting in enhancement of the subsequent reactions. It has been reported that the G protein(s) coupled to the phospholipase C on the exocrine pancreas may be different from G_s or G_i and can be activated by fluoride (7), but the G protein(s) coupled to the phospholipase C has not been identified. To confirm the hypothesis that DCA acts on the coupling of the G protein(s) to the phospholipase C, the identification of the G protein(s) involved in this coupling is necessary. On the other hand, the possibility can not be completely neglected that DCA acts on the phospholipase C-induced hydrolysis of phosphoinositides. Recently, effect of many types of detergents on the phospholipase C activity *in vitro* has been investigated (18-21). It has been well established that DCA stimulates the activity of this enzyme *in vitro* (18-21). However, the concentration of DCA used in *in vitro* stimulation of this enzyme is much higher than that used in our *in vivo* experiment. A low concentration of DCA used in our experiment does not stimulate the phospholipase C activity *in vitro* (data not shown). In addition, DCA alone does not elicit the phospholipase C reaction in our *in vivo* system (1). DCA enhances only the CCK8- or NaF-induced phospholipase C reaction. Thus, it seems less possible that DCA increases the affinity of the phospholipase C to phosphoinositides and enhances the phospholipase C reaction.

In conclusion, DCA acts on the process between the G protein(s) and the phospholipase C reaction and thereby enhances the secretagogue-induced phospholipase C reaction in the pancreatic acini.

REFERENCES

1. Takeyama, Y., Nakanishi, H., Ohyanagi, H., Saitoh, Y., Kaibuchi, K., and Takai, Y. (1986) J. Clin. Invest. 78, 1604-1611.
2. Creutzfeldt, W., and Schmidt, H. (1970) Scand. J. Gastroent. Suppl. 6, 47-62.
3. Ohlsson, K., and Eddeland, A. (1975) Gastroenterology 69, 668-675.
4. Brandt, S.J., Dougherty, R.W., Lapetina, E.G., and Niedel, J.E. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 3277-3280.

5. Cockcroft, S., and Gomperts, B.D. (1985) Nature Lond. 314, 534-536.
6. Lucas, D.O., Bajjalieh, S.M., Kowalchyk, J.A., and Martin, T.F.J. (1985) Biochem. Biophys. Res. Commun. 132, 721-728.
7. Matozaki, T., Sakamoto, C., Nagao, M., Nishizaki, H., and Baba, S. (1988) Am. J. Physiol. 255 (Endocrinol. Metab. 18), E652-E659.
8. Merritt, J.E., Taylor, C.W., Rubin, R.P., and Putney, J.W.Jr. (1986) Biochem. J. 236, 337-343.
9. Moreno, F.J., Mills, I., Garcia-Sainz, J.A., and Fain, J.N. (1983) J. Biol. Chem. 258, 10938-10943.
10. Nakamura, T., and Ui, M. (1985) J. Biol. Chem. 260, 3584-3593.
11. Smith, C.D., Lane, B.C., Kusaka, I., Verghese, M.W., and Synderman, R. (1985) J. Biol. Chem. 260, 5875-5878.
12. Uhing, R.J., Prpic, V., Jiang, H., and Exton, J.H. (1986) J. Biol. Chem. 261, 2140-2146.
13. Gilman, A.G. (1984) Cell 36, 577-579.
14. Ui, M. (1984) Trends Pharmacol. Sci. 5, 277-279.
15. Katada, T., Bokoch, G.M., Northup, J.K., Ui, M., and Gilman, A.G. (1984) J. Biol. Chem. 259, 3568-3577.
16. Sternweis, P.C., and Gilman, A.G. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 4888-4891.
17. Williams, J.A., Korc, M., and Dormer, R.L. (1978) Am. J. Physiol. 235 (Endocriol. Metab. Gastrointest. Physiol. 4), E517-E524.
18. Hofmann, S.L., and Majerus, P.W. (1982) J. Biol. Chem. 257, 6461-6469.
19. Nakanishi, H., Nomura, H., Kikkawa, U., Kishimoto, A., and Nishizuka, Y. (1985) Biochem. Biophys. Res. Commun. 132, 582-590.
20. Shukla, S.D. (1982) Life Sciences 30, 1323-1335.
21. Takenawa, T., and Nagai, Y. (1981) J. Biol. Chem. 256, 6769-6775.