Effects of Dibutyryladenosine 3',5'-Monophosphate on Steroid Biosynthesis in Humans

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Abstract

The effects of dibutyryladenosine 3', 5'-monophosphate (DBcAMP), a nucleotide analogue, on blood pressure, serum electrolytes and plasma corticoid concentrations were investigated in 10 normotensive healthy subjects who received a constant diet containing 5-8 g sodium chloride in hospital. The systolic blood pressure did not change after infusion of 0.25 or 0.33 mg/kg/min of DBcAMP for 20 min. On the other hand, the diastolic blood pressure was significantly decreased after the infusion of DBcAMP.

The levels of serum sodium and potassium were significantly decreased after the infusion of DBcAMP.

After infusion of 0.25 mg/kg/min of DBcAMP for 20 min, the changes in plasma levels of 6 corticoids ((progesterone, deoxycorticosterone (DOC), 18-hydroxy-deoxycorticosterone (18-OH-DOC), corticosterone, cortisol and dehydroepiandrosterone sulfate (DHEA-S)) revealed no significant changes.

After infusion of 0.33 mg/kg/min of DBcAMP for 20 min, the plasma levels of cortisol, corticosterone and 18-OH-DOC were significantly increased and the changes in plasma levels of aldosterone showed a tendency to increase but this was not significant. The plasma levels of DOC and DHEA-S were not appreciably changed, while the plasma levels of progesterone were significantly decreased after the infusion of 0.33 mg/kg/min of DBcAMP.

It is speculated therefore that DBcAMP may act to enhance the activity of the sodium-potassium pump and to promote steroid biosynthesis dose-dependently in humans.

It has been established by many investigators that cyclic AMP, a messenger for information within the cell, plays an important role in adrenal steroid biosynthesis by ACTH (Hynes *et al.*, 1959, Gill, 1972). ACTH promotes cyclic AMP biosynthesis in adrenal cells and cyclic AMP acts to promote adrenal steroid biosynthesis. It has been reported that the mechanism of the increase in steroid biosynthesis due to cyclic

AMP involves the activation of a cyclic AMP-dependent protein kinase to phosphorylate inactive cholesterol ester to release free cholesterol which is a precursor in steroid biosynthesis (Alvano *et al.*, 1974, Beckett *et al.*, 1977).

The nucleotide analogue, dibutyryladenosine 3', 5'-monophosphate (DBcAMP), can cross the cell membrane rather easily compared to cyclic AMP and has similar physiological activities to cyclic AMP (Hasie *et al.*, 1975). There have been several

previous *in vitro* experiments on steroid biosynthesis by DBcAMP using adrenal cell suspension, and the results indicated that DBcAMP acts in provoking steroid biosynthesis (Carr *et al.*, 1981, Balla *et al.*, 1982, Lambert *et al.*, 1983).

In general, the ACTH loading test has been used in the clinical field, in order to evaluate adrenal function. However, the intracellular mechanism after ACTH receptor on adrenal gland cannot be fully evaluated by only ACTH loading test. Therefore, we considered the possibility of utilizing DBcAMP, in order to evaluate intracellular abnormality in steroid biosynthesis.

Furthermore, few reports have dealt with the effects of DBcAMP on the adrenal steroid biosynthesis in humans. The present study was therefore undertaken to elucidate the effects of DBcAMP on adrenal steroid biosynthesis in humans.

Materials and Methods

Normotensive healthy subjects in hospital were used. All subjects received a constant dietary intake containing 5–8 g sodium chloride for a control period of one week. The subjects were divided into two groups. The first group consisted of three male subjects (age 19, 49 and 57 yr). The second group consisted of seven male subjects (age 23–45 yr, mean: 35.4 ± 2.2 (SE) yr). All subjects in both groups were administered dexamethasone (1.0 mg, p.o) at 2300 h on the day before the infusion test and at 0600 h on that day, and enalapril (5.0 mg, p.o) at 0600 h on the day before the test and on that day to inhibit endogenous ACTH and angiotensin II. The DBcAMP infusion tests were performed between 0900 and 1000 h after two hours recumbency. From 0830 h, intravenous infusion of 5% xylitol was begun and at 0900 h DBcAMP dissolved in 100 ml of 5% xylitol was infused intravenously for 20 min. The infusion rate of the DBcAMP was 0.25 mg/kg/min in the first group and 0.33 mg/kg/min in the second group. Blood samples were obtained through an indwelling catheter in the brachial vein before the

beginning of the DBcAMP infusion, and additionally at 20, 40 and 60 min after the DBcAMP infusion.

Blood pressure was measured with an autosphygmomanometer (BP-203N, Nihon Cholin Co., Ltd.). By using each of the blood samples taken, the ACTH, corticosterone and aldosterone were measured using an RIA kit from CEA-IRE-SORIN (France). Plasma progesterone, deoxycorticosterone (DOC), 18-hydroxy-deoxycorticosterone (18-OH-DOC) and dehydroepiandrosterone sulfate (DHEA-S) were determined as reported previously (Honda, 1976). The serum sodium and potassium concentrations were measured by flame photometry. DBcAMP with sodium salt was supplied by Daiichi Pharmaceutical Co. Ltd., Tokyo.

Statistical comparisons were made by Student's *t*-test for paired data and the results were expressed as the mean $\pm$ SE.

Results

The changes in blood pressure and electrolytes before and after the infusion of 0.25 mg/kg/min of DBcAMP for 20 min in the first group are shown in Figure 1. The systolic blood pressure showed an increase in one case, almost no change in one case and a decrease in one case, while the diastolic blood pressure showed decreases in three cases at 20 and 40 min after the infusion. The levels of serum sodium were decreased in three cases at 20, 40 and 60 min after the infusion, and the levels of serum potassium were decreased in three cases at 40 and 60 min after the infusion as shown in Figure 1.

The changes in plasma levels of ACTH and several steroid hormones before and after infusion of 0.25 mg/kg/min of DBcAMP for 20 min in the first group are shown in Figure 2. The plasma levels of ACTH underwent almost no change after the infusion in three cases. The plasma levels of progesterone and DOC were decreased after the infusion. The plasma levels of corticosterone rose slightly in two cases but fell

in one case. The plasma levels of aldosterone were down at 20 and 40 min after the infusion in three cases. The plasma levels of cortisol and DHEA-S did not show any definite tendency after the infusion in three cases. The plasma levels of 18-OH-DHC before and after the infusion were all undetectable (below 0.01 ng/ml, data not shown).

As indicated above, the changes in plasma levels of the seven steroid hormones after the infusion of 0.25 mg/kg/min of DBcAMP for 20 min in the first group did not show any definite tendency. The infusion rate of

DBcAMP was therefore increased to 0.33 mg/kg/min in the second group.

The changes in blood pressure before and after infusion of 0.33 mg/kg/min of DBcAMP in the second group are illustrated in Figure 3. The systolic blood pressure did not change significantly. However, the diastolic blood pressure before and at 20, 40 and 60 min after the infusion was 75.6 ± 6.5 , 56.6 ± 6.3 ($P < 0.001$), 53.1 ± 6.3 ($P < 0.01$) and 57.1 ± 6.1 mmHg ($P < 0.001$), respectively. These responses indicated significant decreases.

The levels of serum sodium before and

Fig. 1. Changes in blood pressure and serum electrolytes before and after infusion of 0.25 mg/kg/min of DBcAMP for 20 min in the first group.

Inf. : Infusion of DBcAMP.

Fig. 2. Changes in plasma levels of ACTH and 6 steroid hormones before and after infusion of 0.25 mg/kg/min of DBcAMP for 20 min in the first group.

Inf. : Infusion of DBcAMP.

S.B.P. and D.B.P.

Fig. 3. Changes in levels of systolic blood pressure (S. B. P.) and diastolic blood pressure (D. B. P.) before and after infusion of 0.33 mg/kg/min of DBcAMP for 20 min in the second group. Vertical bars indicate the mean values $\pm$ SE. The P values (*: $P < 0.01$, **: $P < 0.001$) refer to a comparison with the values before the DBcAMP infusion.

Inf. : Infusion of DBcAMP.

at 20, 40 and 60 min after the infusion were 139.8 ± 1.3 , 137.3 ± 1.5 ($P < 0.001$), 134.0 ± 3.1 ($P < 0.05$) and 134.0 ± 1.2 mEq/l ($P < 0.001$), respectively (Fig. 4). These changes indicated significant decreases. The levels of serum potassium before and at 20, 40 and 60 min after the infusion were 4.38 ± 0.20 , 4.20 ± 0.18 ($P < 0.05$), 4.15 ± 0.17 ($P < 0.02$) and 4.22 ± 0.19 mEq/l ($P < 0.05$), respectively (Fig. 4). These changes also indicated significant decreases.

The plasma levels of ACTH before and at 20, 40 and 60 min after the infusion were 26.0 ± 5.5 , 17.9 ± 3.1 , 15.7 ± 4.9 ($P < 0.02$) and 19.9 ± 5.7 pg/ml ($P < 0.05$), respectively (Fig. 5). These changes represented significant decreases and the lowest level was found at 40 min after the infusion.

The plasma levels of progesterone were decreased from 0.37 ± 0.04 to 0.32 ± 0.04 ($P < 0.05$) and 0.28 ± 0.04 ng/ml ($P < 0.02$) at 40 and 60 min after the infusion, respectively (Fig. 6).

The plasma levels of DOC did not undergo appreciable change after the infusion (Fig. 6). The plasma levels of 18-OH-DOC fell significantly from 0.021 ± 0.005 to 0.031 ± 0.008 ng/ml ($P < 0.05$) at 60 min after the infusion (Fig. 6). The plasma levels of corticosterone before and at 20, 40 and 60 min after the infusion were 1.1 ± 0.2 , 3.0 ± 0.5 ($P < 0.01$), 3.5 ± 0.8 ($P < 0.02$) and 1.9 ± 0.3 ng/ml, respectively (Fig. 6). These changes indicated significant increases at 20 and 40 min after the infusion. The plasma levels of aldosterone before and

Fig. 4. Changes in serum sodium and potassium before and after infusion of 0.33 mg/kg/min of DBcAMP for 20 min. Vertical bars indicate the mean values $\pm$ SE. The P values (*: $P < 0.05$, **: $P < 0.02$, ***: $P < 0.001$) refer to a comparison with the values before the DBcAMP infusion.

Inf. : Infusion of DBcAMP.

Fig. 5. Changes in plasma levels of ACTH before and after infusion of 0.33 mg/kg/min of DBcAMP for 20 min. Vertical bars indicate the mean values $\pm$ SE. The P values (*: $P < 0.05$, **: $P < 0.02$) refer to a comparison with the values before the DBcAMP infusion.

Inf. : Infusion of DBcAMP.

Fig. 6. Changes in plasma levels of progesterone, deoxycorticosterone (DOC), 18-hydroxy-deoxycorticosterone (18-OH-DOC) and corticosterone before and after infusion of 0.33 mg/kg/min of DBcAMP for 20 min. Vertical bars indicate the mean values $\pm$ SE. The P values (*: $P < 0.05$, **: $P < 0.02$, ***: $P < 0.01$) refer to a comparison with the values before the DBcAMP infusion.

Inf. : Infusion of DBcAMP.

at 20, 40 and 60 min after the infusion were 73.9 ± 7.3 , 86.6 ± 6.9 , 88.9 ± 6.7 and 85.6 ± 8.5 pg/ml, respectively (Fig. 7). These changes showed a tendency towards increase at 20 and 40 min after the infusion. The plasma levels of cortisol before and at 20, 40 and 60 min after the infusion were 1.3 ± 0.2 , 2.3 ± 0.3 ($P < 0.01$), 4.2 ± 0.6 ($P < 0.01$) and 3.3 ± 0.5 $\mu\text{g/dl}$ ($P < 0.01$), respectively (Fig. 7). These changes represented significant increases and the level at 40 min after the infusion was the highest. The plasma levels of DHEA-S did not change appreciably after the infusion (Fig. 7).

Discussion

It has been recognized that DBcAMP can cross the cell membrane rather easily and displays similar physiological activities to cyclic AMP. The mechanism of its similar physiological activities has been attributed to the inhibitory effects of DBcAMP

on phosphodiesterase in the cell and furthermore, N⁶-monobutyryladenosine 3', 5'-monophosphate, the metabolic substance of DBcAMP, has a high affinity for the cyclic AMP-binding protein and acts in amplifying the cyclic AMP-dependent protein kinase (Hasie *et al.*, 1975). Many investigators have therefore carried out experiments using DBcAMP in order to clarify the effects of endogenous cyclic AMP in cells.

In the present experiments, the blood pressure, especially the diastolic blood pressure, after infusion of 0.33 mg/kg/min of DBcAMP was significantly decreased rather than that after infusion of 0.25 mg/kg/min of DBcAMP. However, the systolic blood pressure did not change appreciably after infusion of DBcAMP at either a high dose or a low dose. From the present results, it is suggested that the vasodilatic action of DBcAMP may be dose-dependent. Webb *et al.*, (1980) have found that DBcAMP causes an enhancement of potassium-induced relaxation which reflects the activity of the

Fig. 7. Changes in plasma levels of aldosterone, cortisol and dehydro-epiandrosterone sulfate (DHEA-S) before and after infusion of 0.33 mg/kg/min of DBcAMP for 20 min. Vertical bars indicate the mean values $\pm$ SE. The P values (*: $P < 0.01$) refer to a comparison with the values before the DBcAMP infusion.

Inf. : Infusion of DBcAMP.

sodium-potassium pump. In the present experiments, the levels of serum potassium after infusion of DBcAMP were significantly decreased at the same time as a decrease in diastolic blood pressure. It is possible therefore that the decreases in diastolic blood pressure and in levels of serum potassium could be due to amplification of the sodium-potassium pump activity in the cells of the arterial wall and in other cells, respectively. In addition, some reports have indicated that DBcAMP acts in increasing insulin release (Suemori, 1975, Yoshitake *et al.*, 1983). It is therefore possible to explain the decrease in levels of serum potassium, as due to the indirect effects of increased levels of insulin after infusion of DBcAMP.

In the present experiments, the levels of serum sodium were significantly decreased after the infusion of DBcAMP. Some reports have indicated that infusion of DBcAMP into dog artery directly increases natriuresis significantly, and it was suggested that the natriuresis was due to decreases in sodium reabsorption in the proximal tubules through the direct action of DBcAMP on the cells (Gill *et al.*, 1971). It is possible therefore that the present finding of decreases in serum sodium after infusion of DBcAMP was caused with natriuresis by DBcAMP.

As trophic factors to steroid biosynthesis in the adrenal gland, the following can be cited: ACTH, angiotensin II, potassium ion, etc. Among these trophic factors, the effect of ACTH on steroid biosynthesis has been recognized to be dependent on cyclic AMP. Beckett *et al.*, (1977) investigated the mechanism of steroid biosynthesis by cyclic AMP with cortical supernatant. They proposed that an increased level of plasma ACTH stimulates adenyl cyclase to increase the intracellular level of cyclic AMP which activates a cyclic AMP-dependent protein-kinase, and the activated protein-kinase phosphorylates an inactive cholesterol ester hydrolase to an active phosphorylated enzyme. The active cholesterol ester hydrolase is then

able to act on the cholesterol ester in lipid droplets to release free cholesterol. The free cholesterol formed is available for transportation into the mitochondria for cleavage of the side chain to form pregnenolone. Thus, cyclic AMP serves to activate cholesterol ester hydrolase and to provoke steroid biosynthesis. Furthermore, these actions have been recognized in *in vitro* experiments with DBcAMP (Carr *et al.*, 1981, Balla *et al.*, 1982, Lambert *et al.*, 1983), as reported by Beckett *et al.*, (1977). In the present study, we also recognized the effect of DBcAMP in provoking steroid biosynthesis as noted in the *in vitro* experiments.

We had already examined the effects of xylitol on several steroid hormones, serum electrolytes, blood pressure and ACTH with infusion of 500 ml of 5% xylitol for 60 min in patients with essential hypertension and found that the plasma levels of several steroid hormones, serum electrolytes, blood pressure and ACTH did not change significantly. In the present experiments, therefore, it can be considered that the effects of DBcAMP with 5% xylitol are only effects of DBcAMP itself (Ueda *et al.*, 1982).

In the first group of subjects, the plasma levels of cortisol were increased after infusion of 0.25 mg/kg/min of DBcAMP. However, the changes in plasma levels of corticosterone did not reveal a definite tendency and the plasma levels of 18-OH-DOC did not change at all after infusion in this group. On the other hand, after infusion of 0.33 mg/kg/min of DBcAMP for 20 min, the plasma levels of cortisol, corticosterone and 18-OH-DOC were significantly increased. Based on these findings, it appears that DBcAMP may promote the biosynthesis of these steroid hormones dose-dependently. Nevertheless, the plasma levels of aldosterone were decreased after the infusion of 0.25 mg/kg/min of DBcAMP in the first group, whereas after infusion of 0.33 mg/kg/min of DBcAMP, a tendency for the plasma levels of aldosterone to rise was observed.

To explain this discrepancy in the response of plasma aldosterone following two different doses of DBcAMP, the following mechanism is suggested. In the present experiments, the amount of plasma potassium was decreased after the infusion of the low dose as well as high dose of DBcAMP. It is possible therefore that the higher dose of DBcAMP would be needed to stimulate aldosterone biosynthesis, in order to cover the effect of the decreased levels of potassium on aldosterone biosynthesis.

The plasma levels of progesterone, which differed from those of cortisol and corticosterone, were decreased at 40 and 60 min after the infusion. As one possible cause of the decrease in progesterone after the infusion of DBcAMP, it is considered that progesterone, a precursor of several steroid hormones, might be used for the synthesis of cortisol, corticosterone and aldosterone, etc. under the condition of inhibition of synthesis of progesterone by dexamethasone. Interestingly, there is another possibility to explain the decreased levels of progesterone. Several investigators have reported that the plasma levels of free fatty acid are significantly decreased after infusion of DBcAMP and the decrease is due to an enhancement of insulin release by DBcAMP or to secondary changes concomitant with increases in the levels of blood sugar with glyco-genolysis by DBcAMP (Suemori, 1975, Yoshitake *et al.*, 1983). It is possible therefore that, through depression of the plasma levels of free fatty acid and inhibition of the mobilization of cholesterol as materials of progesterone into the adrenal cells, DBcAMP may provoke steroid biosynthesis and, as a result, the plasma levels of progesterone are then decreased.

In the present experiments, the plasma levels of DOC and DHEA-S did not undergo appreciable change after the infusion.

Variations were thus apparent in the synthesis of steroid hormones by DBcAMP. In general, progesterone, DOC and DHEA-S,

steroid hormones at an early step in adrenal steroid biosynthesis, were decreased or did not change, whereas 18-OH-DOC, corticosterone, aldosterone and cortisol, steroid hormones at a late step in adrenal steroid biosynthesis, were increased. These discrepancies are considered to reflect the inhibitory effects of DBcAMP on the mobilization of cholesterol to the adrenal cells as mentioned above as well as variations in sensitivity to DBcAMP among the different steroid hormones.

On the other hand, the plasma levels of ACTH were decreased at 20 and 40 min after infusion of 0.33 mg/kg/min of DBcAMP for 20 min, although ACTH was inhibited by dexamethasone as a premedication. Interestingly, the changes in ACTH and in cortisol and corticosterone were paradoxical. It is possible therefore that the plasma levels of ACTH were decreased by fast, rate-sensitive feedback to cortisol and corticosterone, since the plasma levels of ACTH were decreased already at 20 min after the infusion (Jones *et al.*, 1976).

The steroid biosynthesis by exogenous ACTH in humans may respond through the receptor action of ACTH (Gill, 1972). However, the steroid biosynthesis by DBcAMP does not respond through the receptor action but responds directly to cholesterol ester hydrolase activated by DBcAMP in the adrenal cells (Beckett *et al.*, 1977, Carr *et al.*, 1981). Accordingly, it is considered that comparisons between steroid biosynthesis by ACTH and by DBcAMP in humans may represent a clinically useful technique whereby the receptor abnormality of ACTH and its backward abnormality can be evaluated in the adrenal gland of humans.

In conclusion, the present results suggest that DBcAMP may act to enhance the activity of the sodium-potassium pump and to promote steroid biosynthesis dose-dependently in humans.

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