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IN VIVO DECREASE OF ADENYLATE CYCLASE RESPONSIVENESS TO ISOPROTERENOL IN THE SPONTANEOUSLY HYPERTENSIVE RAT.

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It has recently been reported in rats with different forms of hypertension that adenylate cyclase from cardiac and vascular smooth muscle membranes is less sensitive to isoproterenol activation (1,2,3) and that the number of β-adrenoceptors is reduced (3,4,5,6). In the present study we attempted to demonstrate such alteration of β-adrenergic activation of adenylate cyclase in the intact spontaneously hypertensive rats (SH rats). We measured the plasma level of adenosine 3',5'-monophosphate (cyclic AMP) as a function of isoproterenol dose. This can be considered as an index of β-adrenoceptor stimulation since it has previously been shown that in the rat there is a good correlation between circulating cyclic AMP level and adrenergic activity (7). We report here that the SH rats have a higher basal cyclic AMP plasma level and a lower sensitivity to isoproterenol stimulating cyclic AMP formation than normotensive Wistar and Wistar Kyoto (WKY) rats. These results support the view that adenylate cyclase desensitization occurs in the SH rat as an adaptative mechanism to presumably increased sympathetic activity.

MATERIALS AND METHODS

The experiments were performed in 18 weeks-old male Wistar, Wistar Kyoto and SH (originally from the Okamoto strain) rats which were bred in our animal quarters. Systolic blood pressure (BP) was measured 3 days before the experiment using a tail cuff technique (NARCO Bio-systems). Two days before the experiment a catheter was chronically implanted into the left carotic artery (8) under ketamin anesthesia, and food was withdrawn 24 hrs before the experiment. Isoproterenol was injected subcutaneously and each rat received increasing doses every 30 min. over a period of 4 hrs. When necessary, 0.02 ml blood were withdrawn from the catheter and rapidly mixed in 0.2 ml of an isotonic NaCl solution containing 10 mM EDTA. The presence of EDTA was essential to prevent breakdown of cyclic AMP by phosphodiesterase possibly relased from

platelets. After centrifugation at 4°C, cyclic AMP was assayed in plasma using a radioimmunological micromethod (9).

The maximal effect of isoproterenol (E_{max}) on plasma cyclic AMP level was estimated from the extrapolation of the log-dose function. The dose of isoproterenol producing half maximal response (ED_{50}) was calculated by Probit analysis. These parameters were derived in each rat, using a Digital PDP 11 computer. Statistical analysis was performed using Student's t test.

RESULTS AND DISCUSSION

There was no significant difference in BP between the two normotensive strains (124 ± 3 and 129 ± 2 mmHg in Wistar and WKY rats respectively) in contrast to the significantly elevated BP in the SH rats (178 ± 4 mmHg ; $p < 0.001$ vs Wistar and WKY rats). In Fig. 1 is shown the plasma level of cAMP as a function of administered isoproterenol. Insert A indicates that a plateau of cAMP formation is reached within 20 minutes after administration ; this justifies successive doses at 1/2 hour intervals. Insert B is a magnification of

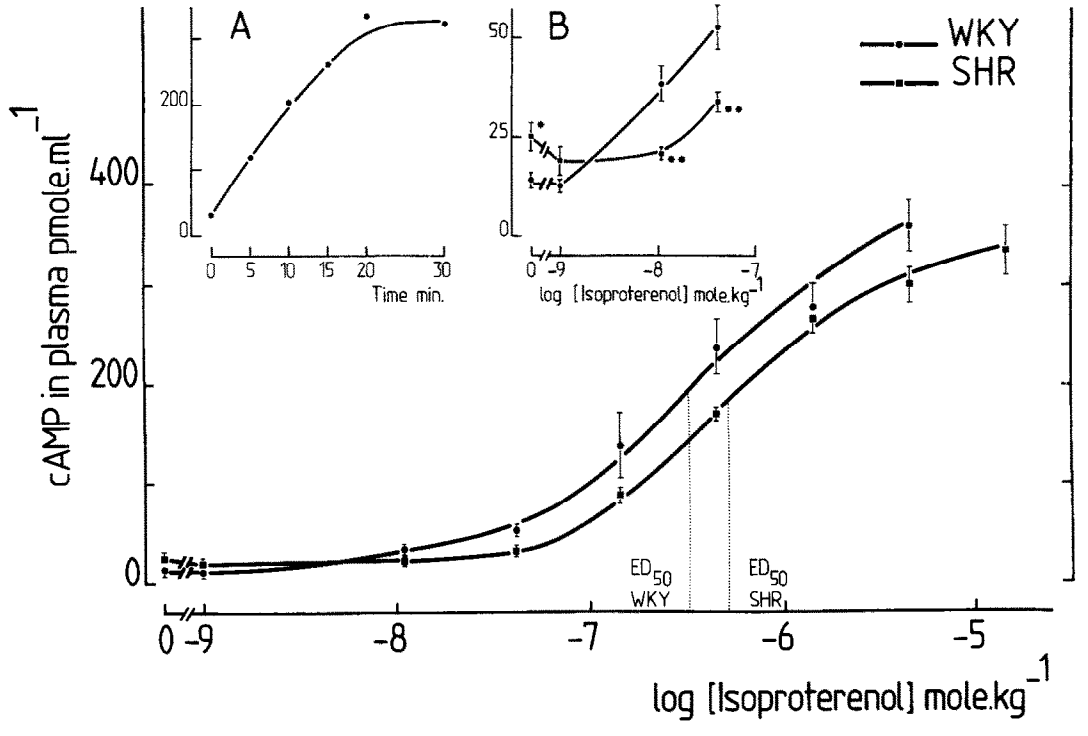

Fig. 1. Effect of subcutaneously injected cumulative doses of isoproterenol on plasma cyclic AMP level in WKY and SH rats. Insert A shows a typical time course of cyclic AMP level after a single injection of 1.10^{-6} mole. kg^{-1} of the agonist in a normotensive Wistar rat. The time course is identical in SH and WKY rats (data not shown). Insert B shows the basal cyclic AMP level and the threshold of isoproterenol in WKY and SH rats. The study of dose-effect relationships was performed on blood samples withdrawn 20 min. after each injection of isoproterenol. Results are the mean of 8 (WKY) or 9 (SH rats) experiments and vertical bars represent S.E.M. $^{*}p < 0.05$; $^{**}p < 0.01$.

the lower end of Fig. 1 clearly demonstrating a significantly higher basal level of plasma cAMP in the SH rats than in the WKY rats. There was no difference between Wistar and WKY rats in basal cyclic AMP level (data not shown).

Over a dose range of 10^{-9} to 10^{-5} mole.kg^{-1} isoproterenol there was no difference in cAMP formation between the two normotensive strains (ED_{50} : 0.33 $\pm$ 0.01 and 0.34 $\pm$ 0.02 µmole.kg^{-1} in Wistar and WKY rats respectively). However, the log dose-effect curve obtained in the SHR was significantly shifted to the righ (ED_{50} : 0.52 $\pm$ 0.03 µmole.kg^{-1} $p < 0.001$ vs Wistar and WKY rats), indicating a subsensitivity of adenylate cyclase in these animals to the stimulatory action of isoproterenol. The finding that differences in plasma cyclic AMP level occured between normotensive and hypertensive rats but not between Wistar and WKY rats suggests that these differences were associated with hypertension rather than strain differences.

The results reported here are consistent with the previous observation of a desensitization of isoproterenol stimulating adenylate cyclase in cardiac and arterial membranes from SH rats (1,2,3). It could be established that this phenomenon is measurable in the intact animal and probably reflects a more extensive tissular response than previously thought, since plasma cyclic AMP levels increased more than 40 times under the influence of isoproterenol.

The basal plasma level of cAMP in SH rats was significantly higher than that in normotensive rats (more than doubled), suggesting that the sympathetic activity in the SH rats was higher than in normotensive rats. Enhanced sympathetic activity has previously been reported in the SH rats and has been implicated in the development and maintenance of hypertension (10, 11). Together with the elevated level of cAMP we observed a decreased responsiveness of adenylate cyclase to isoproterenol as shown by the increase in threshold and the ED_{50} of this drug. Thus, the enhanced sympathetic activity seems to be coupled to a desensitization of adenylate cyclase and therefore represents an adaptative mechanism.

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REFERENCES

1. R.C. Bhalla and T. Ashley, *Biochem. Pharmac.* 27, 1967 (1978).
2. L. Triner, Y. Vuillemoz, M. Vereshy and W.M. Manger, *Biochem. Pharmac.* 24, 743 (1975).
3. R.C. Bhalla, R.V. Sharma and S. Ramanathan, *Biochim. biophys. Acta*, 632, 497 (1980).
4. C. Limas and C.J. Limas, *Biochem. biophys. Res. Commun.* 83, 710 (1978).
5. C.J. Limas and C. Limas, *Biochim. biophys. Acta*, 582, 533 (1979).

6. E.A. Woodcock, Z.W. Funder and C.I. Johnston, *Clin. exp. Pharmac. Physiol.*, 5, 545 (1978).

7. S. Kunitada, M. Honma and M. Ui, *Eur. J. Pharmac.* 48, 159 (1978).

8. G. Waeldele et J.C. Stoclet, *J. Physiol. (Paris)*, 66, 357 (1973).

9. H.L. Cailla, M.R. Racine-Weisbuch and M.A. Delaage, *Analyt. Biochem.* 56, 394 (1973).

10. W.V. Judy, A.M. Watanabe, D.P. Henry, H.R. Besch, W.R. Murphy and G.M. Hockel, *Circ. Res.* 38, II-21 (1976).

11. J.H. Coote and Y. Sato, *Circ. Res.* 40, 571 (1977).