Interaction of Cocaine with Angiotensin and Tyramine on the Isolated Rabbit Aortic Strips

Some recent investigations suggest a possible sympathetic pathway for angiotensin action on the isolated vascular strips. SCHÜMANN and GÜTHER[1] showed from the experiments on the isolated guinea-pig and rat aorta that the angiotensin-produced contraction is reduced to about 30% of the original height by cocaine (2 mg/ml), phentolamine (3 µg/ml) and by repeated doses of tyramine. They also reported that tyramine decreases norepinephrine (NE) content of the tissues to about 50% whereas angiotensin is ineffective on the isolated guinea-pig aortic strips. However, angiotensin caused a decrease to about 43% of NE content of the isolated rat aortic strips. But there are several other reports showing no definite indication that angiotensin causes a release of NE from tissues as observed on the isolated heart muscle[2–4], kidney[2,4] and isolated rabbit aortic strips[5].

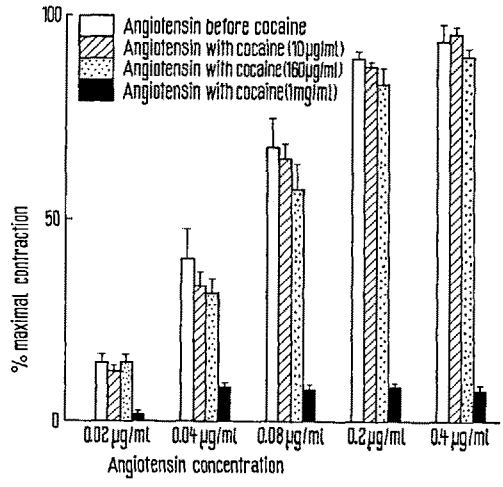
The purpose of the present investigation is to show the effect of cocaine on the contractile action of angiotensin of the isolated rabbit aortic strips in comparison with tyramine, a well-known indirectly acting sympathomimetic amine whose effect is blocked by cocaine.

Material and method. Spirally cut aortic strips of normal and reserpine pre-treated rabbits were prepared according to FURCHGOTT and BHADRAKOM[6]. They were mounted in a 25 ml volume muscle bath in Krebs' solution at 37 °C and aerated with O_2. The lengths and dimension of the strips were kept constant in all experiments and the same tension applied to the strips. Recordings were made isotonically on a smoked paper by a frontal lever with a constant magnification. All results were expressed as % of maximal response determined by adding an excess dose of NE (1 µg/ml) at the beginning and at the termination of each experiments. 5 rabbits were reserpinized using 2.5 mg/kg reserpine the first day, 5 mg/kg the second day and on the third day aortic strips were prepared.

Results. Angiotensin caused a dose-dependent contraction of the isolated strips. This response was neither inhibited nor potentiated by the presence of cocaine (10–160 µg/ml) added into the bath 10 min before angiotensin. However 1 mg/ml of cocaine decreased significantly the contractile effect of angiotensin. These results are summarized in the Figure.

The response to 0.04 µg/ml of angiotensin on reserpine pre-treated strips was 38.0 ± 3.0 S.E.% (n = 5) whereas it was 85.3 ± 2.0 S.E.% (n = 5) for 0.4 µg/ml concentration of the drug. 10–200 µg/ml of cocaine did not inhibit this response. However 1 mg/ml of cocaine caused a significant inhibition of the contractile response of angiotensin on the reserpine pre-treated aorta similar to that of untreated strips. Tyramine (100 µg/ml) alone produced a contractile response of 25.8 ± 1.4 S.E.% (n = 6). This response was partially inhibited (12.4 ± 1.1 S.E.% n = 6) by 5 µg/ml of cocaine and was completely abolished when the concentration of cocaine increased to 10 µg/ml. No response was observed to tyramine on the reserpine pre-treated strips. In another group of experiments, the initial response to angiotensin (0.4 µg/ml) was 80.3 ± 2.0 S.E.% (n = 5) in normal strips, whereas in strips pre-treated with procaine (0.6 mg/ml) it was 20.0 ± 1.5 S.E.% (n = 6). No significant difference was observed in reserpine pre-treated strips. Procaine did not reduce angiotensin responses in both normal and reserpine pre-treated strips when using 10–500 µg/ml concentrations. It also did not change the response to tyramine in concentrations of 10–100 µg/ml. However, the contractile effect of tyramine was reduced when procaine concentration was increased to 100–500 µg/ml.

Discussion. It is well known that tyramine response is mediated by liberating catecholamines from tissues stores. Cocaine (20–40 µg/ml) prevents the tyramine-induced contraction by blocking its NE releasing effect from adrenergic neurons of the vascular wall[7]. However, low concentrations of cocaine cause a release of NE from some tissue stores as described FARRANT[8] and CAMPOS et al.[9]. This also can explain the inhibition of tyramine response when strips are pre-treated with low concentration of cocaine. The present results showed that higher concentrations of cocaine (above 10 µg/ml) blocking the effect of tyramine completely, did not inhibit the contractile action of angiotensin. On the other hand angiotensin responses were not reduced by procaine when adding 10–500 µg/ml in the muscle bath 10 min before angiotensin. However, above these concentrations, procaine also caused significant decrease of angiotensin response in both normal and reserpine pre-treated strips. Both angiotensin and tyramine response in the isolated aortic strips can be blocked by almost the same concentrations of procaine. But this is not the case for cocaine. Cocaine can block the effect of tyramine with low concentrations (5–10 µg/ml) which do not inhibit the contractile effect of angiotensin. On the other hand, previous reserpinization completely abolishes tyramine effect but it does not change angiotensin response on the rabbit aortic strips. These findings strongly support an absence of a sympa-



Dose-response relationship of spirally cut rabbit aorta to angiotensin with and without cocaine. Vertical bar on each column represents standard error of mean in 10 experiments.

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thetic pathway for angiotensin action on the isolated rabbit aortic strips which was described previously[5]. ASTRÖM[10] found that local anaesthetics prevent the response of aortic strips to epinephrine and other stimulants, differently from their local anaesthetic activities. Our present results support ASTRÖM's findings for angiotensin but not for tyramine and cocaine interaction on the isolated rabbit aortic strips[11].

Zusammenfassung. An der isolierten Kaninchenaorta hebt Kokain erst in hoher Konzentration die kontraktile Wirkung von Angiotensin auf. Hingegen wird die Wirkung von Tyramin am selben Präparat in sehr viel niedrigerer Konzentration aufgehoben. Prokain antagonisiert bei gleicher Konzentration die Wirkung der beiden Pharmaka. Die Ergebnisse lassen annehmen, dass eine indirekt sympathomimetische Wirkung von Angiotensin an der isolierten Kaninchenaorta sehr fraglich ist. Ausserdem gibt die gefundene antagonistische Wirkung der verwendeten Lokalanästhetika gegenüber den myotropen Pharmaka den Eindruck, dass dieser Antagonismus unspezifisch ist und nichts mit ihrer lokalanästhetischen Wirkung zu tun hat.

R. K. TÜRKER and E. KARAHÜSEYINOGLU[12]

Department of Pharmacology, Faculty of Medicine, University of Ankara (Turkey), 27 March 1968.

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[12] Student Fellow in the Dept. of Pharmacology, Faculty of Medicine, Ankara University, Ankara (Turkey).

The in vivo Activity of Combinations of 5-Azacytidine and Cytidine on Leukemia L-1210

5-Azacytidine is an antitumor antibiotic which was isolated from a fermentation of *Streptoverticillium ladakanus*[1,2] and also synthesized, independently, by PISKALA and SORM[3]. Preliminary studies have shown that 5-azacytidine is active against the leukemia of AK mice[4,5], T-4 lymphoma[1] and leukemia L-1210[1]. 5-Azacytidine has been shown to inhibit the growth of *Escherichia coli* in a synthetic medium[1] and the inhibition can be reversed by the incorporation of cytidine or uridine into the medium. This paper extends the studies on the anti-leukemic activity of 5-azacytidine and shows that the anti-leukemic activity can be reversed in vivo by either cytidine or uridine.

Materials and methods. 5-Azacytidine, isolated from fermentations of *S. ladakanus*, was obtained from Mr. M. BERGY, The Upjohn Company, and synthetic 5-azacytidine was obtained through the Aldrich Chemical Co. and California Biochemical Corp. Cytidine and uridine were obtained from Nutritional Biochemical Corp.

Female mice ($B6D2F_1/J$), weighing 16–18 g each, were purchased from the Jackson Memorial Laboratories, Bar Harbor, Maine. The leukemia was induced by the i.p. injection of 1.6×10^6 cells obtained from the peritoneal fluid of a donor leukemic mouse. The dosage, route of administration and timing of the injections are given in the Tables. The mice were weighed at the start of each experiment and at intervals thereafter. The mice were checked daily for deaths. The median survival time was calculated for each group.

Results. The most effective dose of 5-azacytidine when given daily for 7 days starting 18 h after implanting the tumor cells was 10 mg/kg · day. This dose is very close to the toxic dose, since 12.5 mg/kg · day produced no increase in survival time and caused a marked loss in body weight during the injection period (Table I).

Studies using the combinations of cytidine and 5-aza-cytidine are shown in Table II. Administration of cytidine 1 h prior to the administration of 5-azacytidine or at the same time but at a different site results in a reduction in the toxicity, as evidenced by weight changes and an increase in the survival time. Administration of cytidine 1 or 2 h after the administration of 5-azacytidine or if the 2 drugs were mixed prior to injection produced no change in either the toxicity of the 5-azacytidine as evidenced by weight changes or survival. Similar observations were made when uridine was substituted for cytidine. From a therapeutic standpoint the combination of 5-azacytidine and either cytidine or uridine has no practical value over the administration of lower doses of 5-azacytidine in daily systemic treatment. The systemic use of either cytidine or uridine may be of value for the protection of the hemopoietic or other susceptible systems when a tumor mass is regionally perfused with 5-azacytidine.

A preliminary experiment indicated that intermittent therapy using relatively high doses of 5-azacytidine was effective in prolonging the survival time. A confirming study in early leukemia (Table III) shows that 20 or 30 mg/kg · day given every 3 days will prolong the survival time 3-fold without too severe loss in body weight. If treatment is delayed until the fifth day (48 h before the first control mouse dies), the treatment is not effective in prolonging the survival time. The concurrent administration of cytidine reduces the loss in body weight during treatment period and the median survival time.

Discussion. The results reported in this paper confirm and extend our work[1] and that of ŠORM[4,5]. We have shown that when the proper dosage regimens are used that a 3-fold increase in survival time can be obtained. It might be expected that if treatment was continued that further extensions of survival time might be expected.

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