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*Report*

# Study on the Renal and Cardiovascular Activities of Aminouracil Derivatives

**Ali A. Mustafa,[1,3] Abdulgader A. Alhaider,[1] and Abdullah A. Hijazi[2]**

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The synthesis of five 4-aminouracil derivatives has been described. These compounds, which are chemically related to xanthines, were tested for possible diuretic activity. The increase in urine output by compounds I, III, and V was comparable to that produced by caffeine in an equimolar dose ($5 \times 10^{-3}$ *M*). On the isolated rabbit's heart (Langendorff's preparation), compounds I, III, and V ($5 \times 10^{-5}$ *M*) significantly increased the amplitude of contractions without having any significant effect on heart rate. Only these three compounds ($4.4 \times 10^{-8}$ *M*) relaxed the isolated rabbit thoracic aorta and rat anococcygeus smooth muscle which were precontracted with norepinephrine ($4 \times 10^{-8}$ *M*). This action was not antagonized by atropine, propranolol, or methylene blue, ruling out the involvement of acetylcholine, beta receptors, or endothelium-derived relaxing factor (EDRF). The relaxant effect, however, was reversed by the addition of calcium chloride, suggesting that this relaxation may be due to inhibition of the entry of extracellular calcium into the cells.

KEY WORDS: diuretic; aminouracil; cardiovascular effects.

## INTRODUCTION

The methylxanthines, caffeine and theophylline, produce a variety of pharmacological activities including bronchodilation, central nervous system stimulation, analgesic potentiation, cardiac stimulation, and diuresis (1). It has been reported that aminophylline produces positive inotropic and chronotropic responses following infusion in the dog, an effect which is markedly reduced by propranolol (2). It was also shown that theophylline potentiates the inotropic response to norepinephrine in isolated atria (3). It has generally been found that methylxanthines cause relaxation of vascular smooth muscles in the presence of various stimulators of contraction such as norepinephrine, angiotensin or potassium (4). The diuretic action of alkylxanthines has been known for many years (5). However, the pharmacological basis for this effect has not been delineated. Since adenosine is known to reduce urine volume and sodium excretion (6) it is possible that adenosine antagonism is responsible for some of the actions of alkylxanthines. Some xanthine analogues which are alkyl derivatives of uracil have been reported (7,8). Furthermore, arylakyl or alkyl derivatives of uracil have also been synthesized by Strauss (9), who suggested that such synthesized compounds might be useful as diuretic and/or vasorelaxant agents. In the present report, some derivatives of 4-aminouracil were synthesized and their possible diuretic, smooth muscle relaxant, and cardiovascular effects were studied.

## MATERIALS AND METHODS

### Chemicals

Norepinephrine bitartrate, atropine sulfate, and propranolol hydrochloride were from Sigma Chemical Company, St. Louis, Missouri, and calcium chloride, caffeine citrate, and melthylene blue were from B.D.H., Poole, Dorset, England.

### Synthesis

The chemical structures and physical properties of five derivatives of uracil are outlined in Table I. The key intermediate, 6-chloro-1,3-dimethyluracil, was prepared from 1,3-dimethylbarbituric acid as the starting material according to the procedure of Pfleiderer and Schundehutte (10). Compounds I–V were prepared by the slow addition of 0.34 mol of the corresponding amine in 20 ml of absolute methanol to 0.05 mol of the 6-chloro-1,3-dimethyluracil in 30 ml of methanol. The reaction mixture was refluxed for 5 hr, then cooled to room temperature. The solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate and refluxed for 15 min. The precipitate was filtered off and washed with 50 ml of ethyl acetate. The filtrate was stirred with 700 mg of NaOH powder for half an hour then filtered to remove NaCl. The filtrate was concentrated to about 20 ml *in vacuo* and kept in a refrigerator overnight. The precipitate was filtered and dried to give the corresponding final product.

Elemental analyses were determined for compounds I–

[1] Department of Medical Pharmacology, College of Medicine and King Khalid University Hospital, King Saud University, P.O. Box 2925, Riyadh 11461, Kingdom of Saudi Arabia.

[2] Department of Chemistry, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia.

[3] To whom correspondence should be addressed.

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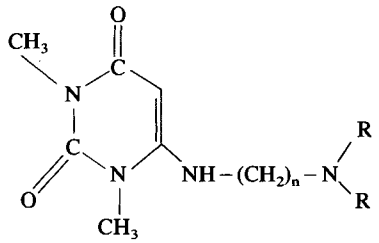
Table I. The Chemical Structures and Analytical Data of Compounds I–V



| Compound | $n$ | $R$ | Elemental analysis C | H | N | Melting point range (°C)[a] | % Yield |
|---|---|---|---|---|---|---|---|
| I | 2 | $CH_3$ | 52.90 (53.08) | 7.88 (8.02) | 24.65 (24.76)[b] | 101–103 | 59 |
| II | 2 | $CH_2CH_3$ | 56.88 (56.67) | 8.97 (8.72) | 22.14[c] (22.03) | 109–111 | 83.5 |
| III | 3 | $-CH_3$ | 54.89 (54.97) | 8.34 (8.39) | 23.31 (23.32) | 101–102 | 79 |
| IV | 3 | $-CH_2CH_3$ | 57.98 (58.13) | 8.89 (9.01) | 21.10 (20.95) | 137–139 | 74 |
| V | 2 | $-CH(CH_3)2$ | 60.59 (60.78) | 9.30 (9.52) | 18.77 (18.90) | 115–117 | 79 |

[a] Melting points were uncorrected and determined on a Metler FP51.
[b] Calculated from the corresponding molecular weight.
[c] Percentages of carbon, hydrogen, and nitrogen are all within 0.4% of the theoretical value.

V and were consistent with the assigned chemical structures (Table I). Spectral data are shown in Table II. Melting points were uncorrected and determined on Metler FP51 and, for compounds I and III, compare favorably with those reported by the authors who originally synthesized them (7,8).

## Pharmacology

### *Urine Output and Electrolyte Concentration Measurements in Rats*

Urine output was measured for groups of male Sprague–Dawley rats weighing 220 ± 10 g randomly selected and housed individually in metabolic cages. Food and water were withheld for 18 hr prior to the start of the experiment, urine was collected for 5 hr, and its volume was measured and analyzed for electrolyte contents. All animals received 50 ml of vehicle (saline) per kg body weight orally. The control group ($N$ = 16) was administered vehicle alone and the treated groups were given the calculated doses of test compounds (Table III). The first group ($N$ = 9) received compound I ($5 \times 10^{-3}$ $M$), the second group ($N$ = 9) was administered compound II ($5 \times 10^{-3}$ $M$), the third group ($N$ = 10) was given compound III ($5 \times 10^{-3}$ $M$), and the fourth and fifth groups ($N$ = 10) were administered compounds IV and V ($5 \times 10^{-3}$ $M$), respectively. Caffeine ($5 \times 10^{-3}$ $M$)

Table II. Spectral Data

| Compound | Spectral data |
|---|---|
| I | IR 3,250, 2990, 2850, 2800, 1700 $cm^{-1}$; NMR (dimethyl sulfoxide-$d_6$): δ 3.25, 3.35 (S, 2×3H, N–$CH_3$), 2.26 [(S, 6H, $N(CH_3)_2$)], 4.78 (S, 1H, CH), 5.42 (br, 1H, NH), 3.10 (m, 2H, NH–$CH_2$), 2.50 (m, 2H, $CH_2$–$CH_2$) |
| II | IR 3250, 2990, 2850, 2800, 1700 $cm^{-1}$; NMR (dimethyl sulfoxide-$d_6$): δ 3.23, 3.1 (S, 2×3H, N–$CH_3$), 2.43 (9, 4H, 2×N–$CH_2$–$CH_3$), 2.6 (m, 2H, NH–$CH_2$–$CH_2$, partially overlapped), 4.16 (S, 1H, CH), 6.4 (br, 1H, NH), 0.95 (t, 6H, 2×N–$CH_2$—$CH_3$) |
| III | IR 3150, 2990, 2850, 2800, 1700 $cm^{-1}$; NMR (dimethyl sulfoxide-$d_6$); δ 3.10, 3.20 (S, 2×3H, N–$CH_3$), 2.16 [(S, 6H, N $(CH_3)_2$)], 4.58 (S, 1H, CH), 7.20 (br, 1H, NH), 3.10 (m, 2H, –NH–$CH_2$) 1.65 (t, 2H, $CH_2$–N), 2.25 (t, 2H, –$CH_2$–) |
| IV | IR 3380, 3150, 2980, 2850, 2800, 1720 $cm^{-1}$; NMR (dimethyl sulfoxide-$d_6$); δ 3.1, 3.35 (S, 2×3H, N–$CH_3$), 2.9–3.3 (m, 8H, $CH_2$–NH, $CH_2$N, 2×N–$CH_2$–$CH_3$), 4.8 (S, 1H, CH), 1.25 (t, 6H, 2×N–$CH_2$ $CH_3$), 1.95 (m, 2H, $CH_2$–$CH_2$–$CH_2$), 7.00 (br, 1H, NH) |
| V | IR 3250, 2990, 2860, 2800, 1710 $cm^{-1}$; NMR (dimethyl sulfoxide-$d_6$); δ 3.2, 3.3 (S, 2×3H, N–$CH_3$), 2.6–3.1 (complex signal, 6H, NH–$CH_2$–$CH_2$–N, N–CH/–CH), 0.95 [(d, 12H, 2×N–CH $(CH_3)_2$)], 4.7 (S, 1H, CH), 6.5 (br, 1H, NH) |

Table III. Effect of Caffeine, Compounds I to V, and Saline on Urine Volume and Electrolyte Excretion in Rats

| Treatment | N | Urine volume (ml/100 g)/(5 hr) | (mequiv/100 g)/(5 hr) | | |
|---|---|---|---|---|---|
| | | | $Na^+$ | $K^+$ | $Cl^-$ |
| Saline (5 ml/100 g) (p.o.) | 16 | 3.18 ± 0.13 | 0.44 ± 0.05 | 0.15 ± 0.03 | 0.44 ± 0.02 |
| Caffeine ($5 \times 10^{-3}$ *M*) (p.o.) | 8 | 4.35 ± 0.33* | 0.58 ± 0.02* | 0.19 ± 0.02* | 0.59 ± 0.01** |
| Compd I ($5 \times 10^{-3}$ *M*) (p.o.) | 9 | 4.15 ± 0.01* | 0.62 ± 0.03* | 0.23 ± 0.02* | 0.57 ± 0.01** |
| Compd II ($5 \times 10^{-3}$ *M*) (p.o.) | 9 | 2.85 ± 0.30 | 0.41 ± 0.06 | 0.13 ± 0.02 | 0.42 ± 0.05 |
| Compd III ($5 \times 10^{-3}$ *M*) (p.o.) | 10 | 4.46 ± 0.22* | 0.59 ± 0.01* | 0.24 ± 0.01* | 0.58 ± 0.015** |
| Compd IV ($5 \times 10^{-3}$ *M*) (p.o.) | 10 | 2.95 ± 0.02 | 0.43 ± 0.05 | 0.14 ± 0.01 | 0.42 ± 0.01 |
| Compd V ($5 \times 10^{-3}$ *M*) (p.o.) | 10 | 4.60 ± 0.03* | 0.61 ± 0.02* | 0.26 ± 0.02* | 0.60 ± 0.01** |

* $P < 0.05$ relative to saline control.
** $P < 0.02$ relative to saline control.

was administered orally to another group ($N = 8$) and used as a standard xanthine derivative. Sodium, potassium, and chloride were determined in urine samples using a flame photometer (Eppendorf FCM 6341, West Germany), while chloride was analyzed with a Eppendorf chloride meter (Eppendorf 660, West Germany).

*Isolated Rabbit Aortic Strips*

New Zealand white rabbits of either sex weighing between 2 and 2.5 kg were killed by a blow on the head and then bled from the carotid artery and the thoracic aorta was quickly excised. Aortic spiral strips 2.5 mm in width and 30 mm in length were prepared according to the method of Furchgott and Bhadrakom (11). The spiral strips were mounted in a 10-ml bath containing Krebs solutions (pH 7.4) of the following composition (m*M*): NaCl, 115.0; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25.0; $KH_2PO_4$, 1.2; dextrose, 10.0; aerated with a mixture of oxygen (95%) and carbon dioxide (5%) at 37°C. The responses of the aortic strips were recorded isometrically using a Narco force displacement transducer and a Narco MK-III recorder. Before starting the experiment, strips were allowed to equilibrate for 1 hr in the bathing solution and the resting tension was adjusted to 2 g with solution replacement every 15 min. A submaximally effective concentration of norepinephrine was added two or three times until successive responses remained constant.

*The Anococcygeus Muscle of the Rat*

Male Sprague–Dawley rats were stunned and exsanguinated. The anococcygeus muscle was set according to the method of Gillespie (12). Experiments were performed in the presence of a modified Krebs solution of the following composition (m*M*): NaCl, 116; KCl, 5.4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaH_2PO_4$, 1.2; $NaHCO_3$, 22.0; glucose, 11.2; equilibrated with a mixture of oxygen (95%) and carbon dioxide (5%) at 37°C. Isometric contractile responses were recorded with a Narco force displacement transducer and displayed on a physiograph (Narco MK-III). Individual anococcygeus muscles were mounted under 1.0 g tension in 10-ml organ baths containing Krebs solution. Calcium chloride (0.5 m*M*) was added to Krebs solution of each tissue for 15 min before the addition of the compounds under investigation.

*Isolated Perfused Rabbit's Heart (Langendorff's Preparation)*

New Zealand white male rabbits were killed by a blow on the head and the heart with at least 1 cm of aorta attached was quickly removed. The aorta was located, dissected free of other vessels, and tied to the glass cannula of the perfusing apparatus. A thread attached to the ventricle by a hook was connected to a force displacement transducer (Bio Science Type D) and contractions were recorded on a Bio Science Washington 400 MD 2R recorder.

**Statistics**

Results are expressed as the mean ± SE. Analysis of variance was performed to compare all groups. When significant, the Newman–Keul method for multiple comparison was used to find the significantly different groups.

## RESULTS

**Effect on Urine Output and Electrolyte Excretion**

Acute treatment with compounds I to V at either $5 \times 10^{-6}$ or $5 \times 10^{-4}$ *M* did not affect the urine output by rats. However, at a dose of $5 \times 10^{-3}$ *M* compounds I, III, and V increased the output of urine following oral administration (Table III). The cumulative urine collected over a period of 5 hr was significantly increased compared to that of rats which received saline only ($P < 0.05$) for compounds I, III, and V. Similarly, the oral administration of caffeine ($5 \times 10^{-3}$ *M*) caused a significant increase in urine output in comparison with saline controls ($P < 0.05$). The effect of oral administration of compounds I to V ($5 \times 10^{-3}$ *M*) on electrolyte excretion in urine of rats is shown in Table III. Compounds I, III, and V ($5 \times 10^{-3}$ *M*) caused a significant increase in sodium, potassium, and chloride excretion in urine of treated rats as compared to saline controls. The effect of the three compounds is comparable to that produced by an equimolar dose of caffeine.

### Effect on Rat Anococcygeus Muscle and Rabbit Aortic Strips

Compounds I to V ($4.4 \times 10^{-8}$ *M*) had no effect on the rat's anococcygeus muscle at resting tone. Compounds I, III, and V, however, caused a significant relaxation of the smooth muscle when it was precontracted with norepinephrine ($4 \times 10^{-8}$ *M*). Figure 1a shows a representative trace of the relaxation produced by compound I ($4.4 \times 10^{-8}$ *M*). Calcium chloride (0.5 m*M*), added to the bathing medium 15 min prior to compound I, completely reversed the relaxation produced by a dose ($4.4 \times 10^{-9}$ *M*) of compound I (Fig. 1b). Figure 1c shows the recovery of the contractile response produced by norepinephrine following washing of both compound I and calcium chloride. Compound II or IV or caffeine at an equimolar dose ($4.4 \times 10^{-8}$ *M*) caused no significant relaxation of the muscle (Table IV).

Similarly, the 4-aminouracil derivatives (compounds I, III, and V) relaxed the isolated rabbit aortic muscle precontracted with norepinephrine ($2 \times 10^{-8}$ *M*). This relaxant effect was not antagonized by $10^{-6}$ *M* atropine (*N* = 6), $10^{-5}$ *M* propranolol (*N* = 5), or 3.5 mg/ml methylene blue (*N* = 5). Caffeine at an equimolar concentration to the compounds ($4.4 \times 10^{-8}$ *M*) caused no relaxation of the isolated rabbit aortic strip.

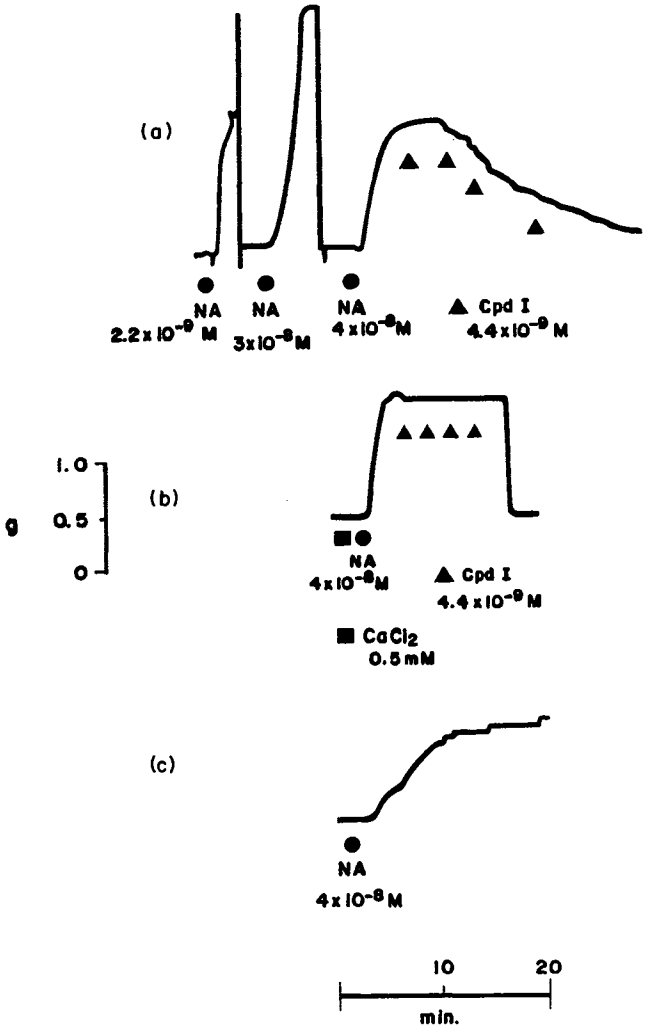



Fig. 1. The smooth muscle relaxant effect of cumalative doses of compound I ($4.4 \times 10^{-9}$ *M*) on rat anococcygeus muscle precontracted with NA ($4 \times 10^{-8}$ *M*). (a) The reversal of this relaxation by the previous addition of calcium chloride (0.5 m*M*) to the bath (b) and the recovery from these effects of compounds I and calcium chloride following several washings of the preparation (c).

Table IV. Effect of Compounds I to V and Caffeine ($4.4 \times 10^{8}$ *M*) on Rat Anococcygeus Muscle and the Isolated Aortic Strip of the Rabbit

| Treatment | Conc. (*M*) | *N* | Rat anococcygeus muscle, tension (g) developed by NA ($4 \times 10^{-8}$ *M*) | Rabbit aortic strip, tension (g) developed by NA ($2 \times 10^{-8}$ *M*) |
|---|---|---|---|---|
| Saline | — | 6 | 1.25 ± 0.04[a] | 1.65 ± 0.06 |
| Caffeine | $4.4 \times 10^{-8}$ | 6 | 1.18 ± 0.06 | 1.42 ± 0.09 |
| Compd I | $4.4 \times 10^{-8}$ | 8 | 0.52 ± 0.01* | 0.68 ± 0.03* |
| Compd II | $4.4 \times 10^{-8}$ | 8 | 1.09 ± 0.02 | 1.51 ± 0.04 |
| Compd III | $4.4 \times 10^{-8}$ | 9 | 0.63 ± 0.02* | 0.79 ± 0.02* |
| Compd IV | $4.4 \times 10^{-8}$ | 9 | 1.11 ± 0.03 | 1.48 ± 0.08 |
| Compd V | $4.4 \times 10^{-8}$ | 8 | 0.78 ± 0.07* | 0.92 ± 0.05* |

[a] Mean ± SE.
* $P < 0.05$ relative to saline control.

### Effects on the Isolated Perfused Rabbit's Heart

At a relatively low dose of the 4-aminouracil derivatives ($2.5 \times 10^{-6}$ *M*), the amplitude and rate of the isolated rabbit's heart were not significantly changed. However, at a relatively higher concentration ($5 \times 10^{-5}$ *M*) compounds I, II, and V produced a significant increase in the amplitude of heart contractions ($P < 0.05$) compared to saline controls. Similarly, caffeine ($5 \times 10^{-5}$ *M*) produced a significant increase in the amplitude and rate of contractions as compared to saline control (Table V).

Table V. The Effect of Caffeine and Compounds I to V on the Isolated Rabbit's Heart (Langendorff's Preparation)

| Treatment | Dose (*M*) | *N* | Change in cardiac function: Rate | Change in cardiac function: Amplitude |
|---|---|---|---|---|
| Saline | — | 6 | 100.0 | 100.0 |
| Compd I | $2.5 \times 10^{-6}$ | 4 | 102.3 ± 7.6 | 115.0 ± 9.1 |
| | $5 \times 10^{-5}$ | 5 | 100.0 ± 8.6 | 136.4 ± 10.2* |
| Compd II | $2.5 \times 10^{-6}$ | 4 | 105.3 ± 8.1 | 110.0 ± 9.3 |
| | $5 \times 10^{-5}$ | 6 | 100.0 ± 9.7 | 139.9 ± 10.1* |
| Compd III | $2.5 \times 10^{-6}$ | 5 | 97.7 ± 8.2 | 118.2 ± 10.1 |
| | $5 \times 10^{-5}$ | 6 | 100.0 ± 9.4 | 119.0 ± 9.4 |
| Compd IV | $2.5 \times 10^{-6}$ | 4 | 102.3 ± 9.1 | 123.8 ± 9.9 |
| | $5 \times 10^{-5}$ | 6 | 97.6 ± 6.2 | 114.4 ± 8.2 |
| Compd V | $2.5 \times 10^{-6}$ | 5 | 100.0 ± 7.8 | 140.0 ± 12.1 |
| | $5 \times 10^{-5}$ | 6 | 107.1 ± 11.1 | 166.6 ± 7.9 |
| Caffeine | $2.5 \times 10^{-6}$ | 6 | 100.5 ± 8.1 | 108.6 ± 6.5 |
| | $5 \times 10^{-5}$ | 6 | 148.4 ± 5.2** | 149.3 ± 5.3* |

* $P < 0.05$ relative to the response evoked by the saline control.
** $P < 0.01$ relative to the response evoked by the saline control.

## DISCUSSION

The results of the present study show that some of the 4-aminouracil derivatives (compounds I, III, V) increase urine output in rats in a manner similar to that of caffeine. They also increased the excretion of sodium, potassium, and chloride in voided urine of rats. These results corroborate the finding that methylxanthines in certain clinical conditions were shown to cause sodium, potassium, and chloride loss in urine (13).

Epinephrine-induced contractions of smooth muscles and of vascular beds in particular were shown to consist of a fast and a slow phase, with the slow plateau phase more dependent on extracellular calcium (14–16). Three of the uracil derivatives (compounds I, III, and V) produced relaxation of both isolated rabbit aorta and rat anococcygeus muscles. This relaxation was not affected by atropine, propranolol, or methylene blue ruling out the involvement of acetylcholine, beta-adrenoceptors, or the endothelium-derived relaxing factor (EDRF) (11), respectively, in the mediation of this response. As this effect was reversed by the addition of calcium chloride, it is likely that the 4-aminouracil derivatives produced this relaxation by blocking the entry of extracellular calcium into the smooth muscle. However, the precise mechanism by which these derivatives affect calcium translocation remains to be determined.

The present study also demonstrates that the 4-aminouracils do not affect the contractility or rate of the isolated rabbit's heart at low doses ($2.5 \times 10^{-5}$ *M*). However, a higher dose ($5 \times 10^{-5}$ *M*) of the compounds (except compounds II and IV) produced a significant inotropic effect on the Langendorff's preparation. Theophylline was shown to increase heart rate concomitant with an increase in the concentration of epinephrine in plasma of human subjects (17), an effect which was blocked by propranolol. This suggests that the positive inotropic effect produced by theophylline may, at least in part, be mediated through stimulation of beta-adrenoceptors. Additionally, the positive inotropic effect of theophylline infusion in the dog has been reported to be mediated through catecholamine release (4). Since it has been suggested that methylxanthines may act by reducing the uptake of catecholamines into neuronal tissue (18), it is also possible that compounds I, III, and V may have acted similarly to increase myocardial contractility.

The present study demonstrates that compounds I, III, and V have a diuretic and a saluretic effect. This effect is probably related to antagonism by these aminouracils of adenosine receptors. This hypothesis is supported by the finding that analogues of the xanthine theophylline produce diuresis and saluresis by antagonizing these receptors (19).

In conclusion, of the five aminouracil derivatives studied, only compounds I, III, and V increased significantly the output of urine with a concomitant increase in electrolyte excretion. All compounds relaxed the vascular smooth muscle (i.e., rabbit aorta) and compounds I, III, and V have also produced a positive inotropic effect on the heart.

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