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EFFECTS OF GANGLIONIC BLOCKADE AND ADRENALECTOMY ON THE CARDIO-RESPIRATORY RESPONSE TO COW'S URINE CONCOCTION IN RATS

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Summary

The cardio-respiratory effects of cow's urine concoction (CUC) were studied in anaesthetized normal, hexamethonium-treated and adrenalectomized rats. The results showed an early response characterized by a transient depressor effect followed by a more sustained pressor effect. This was followed by a late depressor response. Hypotension was most marked in the adrenalectomized group. The cardio-respiratory effects of CUC in the normal and hexamethonium-treated groups, were milder than in the adrenalectomized group. Animals in these two groups survived the effects of CUC better than the adrenalectomized animals. The response of sham-operated controls showed that the greater effects of CUC in the latter group were not the sequelae of surgical intervention. Further investigation revealed that the secretion of catecholamines from the adrenal glands is indispensable in the resistance of the animals to the toxic effects of CUC. It was also established that in CUC-induced death, respiratory failure precedes circulatory failure. However, failures of both respiration and circulation were the causes of death from CUC.

Résumé

On a étudié les effets cardio-respiratoires de la mixtion d'urine de vache (MUV) chez des rats anesthésiés normaux, traités à l'hexaméthonium et adrénalectomisés. Les resultats ont montré une réaction characterizé du vaso-constrictrice transitoire suivi d'une reaction plus prolongé. L'hypotension a été le plus marquée chez le groupe adrenalectomisé. Les effets de MUV ont été moindres chez le groupe normal et le group traité a l'hexaméthonium que chez le groupe adrénalectomisé. Il est évident que les effets de MUV ne sont pas dus aux intervention chirurgicale. Les recherches ulterieures ont montré que les sécrétions de la région médullaire de la glande – les catécholamines – sont indispensables dans la résistance des animaux aux effets toxiques de MUV. On a également établi que dans la mort induite par MUV, la défaillance respiratoire précéde la circulatoire. Neanmoins, les deux défaillances ont été cause de la mort induite par MUV.

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Introduction

Cow's urine concoction (CUC) is the commonest cause of poisoning in children in certain parts of Nigeria (Familusi & Sinnette, 1971). Previous experiments in animals showed that CUC has pressor effects when administered to cats and dogs; it also caused increases in heart rate, cardiac output and marked depressive effects on respiration (Elegbe,

Bamgbose & Oyebola, 1976; Elegbe & Oyebola, 1977). Cow's urine concoction has also been reported to cause increased myocardial contractility (Ojewole, 1979). Elegbe *et al.* (1976) showed that pre-treatment of experimental animals with the α-receptor blocker, phenoxybenzamine, abolished the pressor response to CUC. The latter observation provided strong evidence that the catecholamines are involved in mediating some of the effects of CUC. The two possible endogenous sources of such catecholamines are the adrenal medulla and the sympathetic post-ganglionic nerve endings.

Cow's urine concoction contains nicotine which is present in *Nicotiana tabacum*, one of the ingredients used for preparing CUC (Oyebola & Elegbe, 1975). The ganglion-stimulating effect of nicotine and its ability to cause discharge of epinephrine from the adrenal medulla are well documented (Westfall, 1965; Armitage & Milton, 1965). The relative contribution of the catecholamines released from adrenergic sympathetic post-ganglionic nerve endings and that from the adrenal medulla to the overall effects of CUC is unknown. The effect (if any) of adrenal cortical secretions is also unknown. This study was carried out to investigate the effects of ganglionic blockade and bilateral adrenalectomy on the cardio-respiratory response to CUC.

Materials and methods

Experiments were carried out on three groups of male albino Wistar rats weighing 240–300 g. Group I (twelve rats) served as the control (untreated). Group II (twelve rats) was treated with hexamethonium bromide and group III (ten rats) was adrenalectomized before being used for the experiments.

Hexamethonium-treated group

Anaesthesia was induced with pentobarbital sodium 36 mg/kg given intraperitoneally, with supplemental doses given intravenously (i.v.) as required to maintain light anaesthesia. The trachea was cannulated to allow free breathing from room air. A carotid artery was cannulated and connected via a transducer (Nihor-Kohden, Japan; type MPU – 0.5I) to a polygraph for blood-pressure recording. A femoral vein was cannulated for i.v. injections. Electrocardiogram (ECG, Lead II) was recorded using a biophysical input panel (type RBL-45, Nihon-Kohden, Japan). Each rat was given i.v. 100 units (1 mg) of heparin/100 g body weight and a supplemental dose of 50 units hourly to maintain fluidity of blood in the cannulae. Amplitude of respiratory movement (RA) was monitored via a silk loop attached along the midline of the ventral aspect of the thorax and abdomen at two points about 2 cm apart on the wall of the upper abdomen and lower thorax. This was the area of maximal respiratory movement on the body wall. This silk loop was attached with a string to a force/displacement transducer (type SB-IT; Nihon-Kohden, Japan) using the displacement mode coupled to a carrier amplifier model R.P.-5. Each animal was given hexamethonium bromide (Sigma, London) 5 mg/kg body weight i.v. Twenty min was allowed for the drug to take effect. Blood pressure (BP), ECG and RA were continuously recorded on a multi-purpose 4-channel ink-writing, polygraph (model RM-46, Nihon-Kohden).

Adrenalectomized group

Anaesthesia was induced and maintained by 'open-drop' ether inhalation. Using aseptic techniques, acute bilateral adrenalectomy was performed in each rat using a dorsal approach (Griffith & Parris, 1942). The incision wound was closed in layers. The drinking water of the animals was substituted with 1% saline solution fresh daily throughout the post-operative period. Two rats out of twelve died during surgery, presumably from anaesthetic overdose. All the ten rats that survived the operation recovered fully and were used for the experiments after 4–5 days recovery. Each animal was then set up for the recording of BP, RA and ECG as above. A group of six rats was sham-operated and used as control for the adrenalectomized group. The adrenal glands in the sham-operated group were exposed as above. The glands were gently massaged, but not removed. The animals were then closed up and also given 4–5 days to recover. They were then set up for recording of BP, RA and ECG.

Untreated (control) group

The animals were set up for experiment as in the hexamethonium group but were not given any pre-treatment.

Source of CUC

Full preparation of CUC was prepared in the laboratory using the method of Oyebola and Adetuyibi (1977) 4 weeks before commencement of experiments. Previous studies have established that CUC has a shelf-life of well over 2 years (Elegbe & Oyebola, 1977).

Experimental procedure

In group I, after a 30 min recovery period from surgery, basal (resting) recordings of BP, RA and ECG were made for 10 min at a paper speed of 25 mm/min. At the end of each 5 min slow run, a fast run at 50 mm/sec was carried out for 3 sec to allow easy reading of respiratory rate (RR), ECG pattern and heart-rate (HR) from the ECG.

The animals were then given 0.2 ml/100 g body weight of undiluted CUC slowly i.v. Recording of BP, ECG and RA were continued at 25 mm/min for 70 min post-injection, with fast runs every 5 min as indicated above.

The above procedure was repeated in the hexamethonium-treated and adrenalectomized groups.

0.15 M saline in volumes equivalent to those of CUC used was given as control injections in each group.

The effects of CUC were more profound in the adrenalectomized animals. On the basis of our earlier findings (Elegbe *et al.*, 1976), loss of catecholamines from the adrenal medulla was considered a possible cause of the greater susceptibility to the toxic effects of CUC, especially the profound hypotension. In order to test the latter, five more rats were bilaterally adrenalectomized and set up for recording. This group was given simultaneous injections of CUC and adrenaline (1 μg/kg).

Calculations

The BP, HR and RA were read at 5 min intervals from the tracings and their means (± s.e.) were calculated. The mean BP was computed from the systolic and diastolic values.

Assuming that other variables in the mechanics of breathing remain constant, lung volume (V) is proportional to RA (chest expansion). The product of RR and RA will therefore give an index of the pulmonary ventilation rate (IPVR) per min. Since the resting tension in the silk thread used for monitoring RA was not standardized for the animals studied, the percentage changes in the product of RR and RA after injection of CUC compared with the pre-injection values were taken as a more reliable measure of the absolute change in the IPVR. The latter values were calculated from the 5 min interval observations of RR and RA in groups I, II and III. The mean IPVR (± s.e.) were plotted.

The mean values of the parameters measured (± s.e.) at corresponding 5 min intervals between the three groups studied were tested for statistically significant differences using the *t*-test of difference between two independent sample means (Bahn, 1972). Also, mean values of parameters measured before and in successive 5 min after injection in the same group were assessed for statistically significant differences using the *t*-test for paired data. In all cases, *P* values of 0.05 or less were taken as statistically significant.

Results

All the animals studied survived the effects of the first injection of CUC at the volumes used in this study. All the adrenalectomized animals, however, died when given a second injection of CUC whereas many animals in groups I and II and the sham-operated animals survived a second and sometimes a third injection of CUC. However, in the preliminary experiments, some animals died from overdosage of CUC before the non-lethal volume was established. In all the animals that died, either from overdosage from a first injection or from a second or third injection of a non-lethal first dose, respiratory arrest occurred first. This was then followed by a rapid collapse of the circulation. This pattern was observed consistently in all the animals that died from CUC toxicity.

Effects of CUC on blood pressure (BP)

The effects of CUC on BP are as shown in Table 1 and Figs 1 and 2. Figure 1 shows a typical early BP effect of CUC in a normal animal. The early effect is characterized by a transient fall in BP followed by a marked pressor response lasting about 2 min (Fig. 1). The late effect is characterized by progressive hypotension which persists for 10–20 min post-injection in some animals. Thereafter, blood pressure returned gradually to resting values (Fig. 2). The early and late effects of CUC on BP in groups I, II and III are shown in Table 1. The hypotension following CUC administration was most profound in group III. In Table 1, post-injection BP values that are significantly different from control values in each group are asterisked. Blood-pressure values in group I are significantly greater than in group II from the 10th to the 70th min post-injection ($P<0.05$). Also, BP values in group I are significantly greater than in group III from the 5th to the 70th min post-injection (P values are between 0.001 and 0.05).

Effects of CUC on heart rate (HR) (see Table 2)

Heart rate was first reduced after CUC injection in all the groups. The HR returned to pre-injection values later in the recovery period. Again, the reduction in HR in group III was the most profound while group II occupied an intermediate position. Reduction in HR in group III is significantly lower than in group I in the 5th, 10th and 20th min post-injection ($P<0.05$). All other between-group comparisons of corresponding values are not significant.

Effect of CUC on respiration (see Table 3)

In group I, CUC caused a progressive increase in respiration. These increases were significant in the 50th, 60th, 65th and 70th min post-injection. The response in group II was similar to group I. The increases observed in group II were, however, not significant when compared with the control for the group. In group III, apart from an initial significant reduction in RR, the increases in RR observed in groups I and II later in the recovery period were absent. Five min values of RR in group III are significantly lower than corresponding values in group I between the 10th and 70th min post-injection ($P<0.05$).

All other between-group comparisons of corresponding values are not significant.

The amplitude of respiratory excursion (RA) increased by 120% in the first 2 min of injection in the untreated group but this increase fell to about 10% within 30 min post-injection. After this, an increase in RA of between 4 and 10% was maintained for the remaining period of observation. In the hexamethonium-treated group, the peak increase in RA was 70%. This fell to 2–10% in the last 25 min of post-injection observation. In the adrenalectomized animals, there was an initial decrease of 30% in RA. Except for a spike of increases of about 60 and 40% in the 5th and 40th min post-injection, respectively, RA in the adrenalectomized animals was an average of 20% less than the resting value.

When the percentage increase in pulmonary ventilation (IPVR) were computed from the product of the RR and RA, the pattern that emerged was as shown in Fig. 3. Pulmonary ventilation in groups I and II showed a marginal increase while the net effect in group III was a slight reduction. The IPVR values in group III were significantly lower than those for group I in most cases ($P<0.05$).

Effect of CUC on ECG

The ECG pattern became bizarre after administration of CUC in the three groups. In group I, blunting of the P wave, tall QRS complex and elevated T waves were observed. In group II there was loss of P and T waves. The latter were sometimes inverted. Initial tall QRS complex was soon followed by a stunted QRS pattern. The ECG changes were more marked within the first 2 min of CUC injection. Group III was characterized by reduced ECG voltage, bradycardia, loss of P and T waves and occasional inverted T waves.

TABLE 1. Blood pressure (mmHg) in groups I, II and III before and after CUC injection

	Post-injection period (min)										
	Control	Initial fall	Early rise	5	10	15	20	25	30	50	70
Group I (untreated)	126.8 ± 2.1	74.7* ± 11.4	153.5* ± 6.6	88.1* ± 6.8	111.2 ± 7.6	116.7 ± 9.4	120.0 ± 10.1	121.8 ± 9.7	122.8 ± 9.0	121.9 ± 7.5	121.7 ± 6.7
Group II (hexamethonium-treated)	105.4 ± 4.8	–	181.3* ± 4.7	82.0 ± 8.2	85.0 ± 9.3	93.8 ± 7.1	101.7 ± 7.6	105.8 ± 6.9	107.5 ± 6.7	107.0 ± 5.7	107.9 ± 5.8
Group III (adrenal-ectomized)	110.8 ± 6.0	75.0 ± 2.6	140.6 ± 7.6	35.2* ± 2.7	37.7* ± 4.2	49.1* ± 6.2	61.1** ± 12.6	70.2** ± 16.0	82.8 ± 16.3	82.5 ± 15.8	90.8 ± 15.2

In Tables 1, 2 and 3, time is in min from the moment of injection and * = $P<0.01$; ** = $P<0.05$.

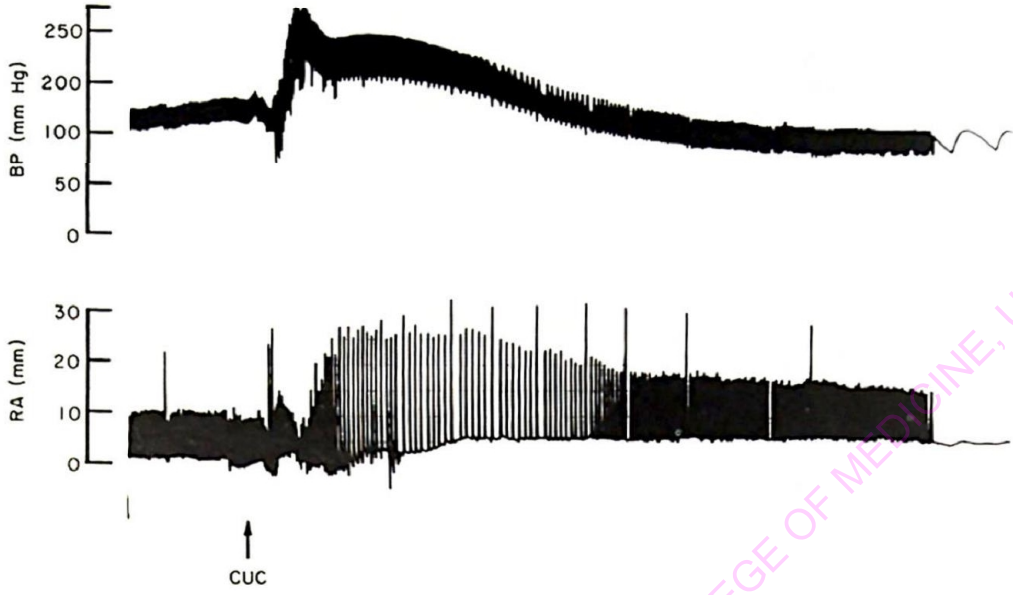

FIG. 1. A typical early BP and respiratory amplitude (RA) response to CUC.

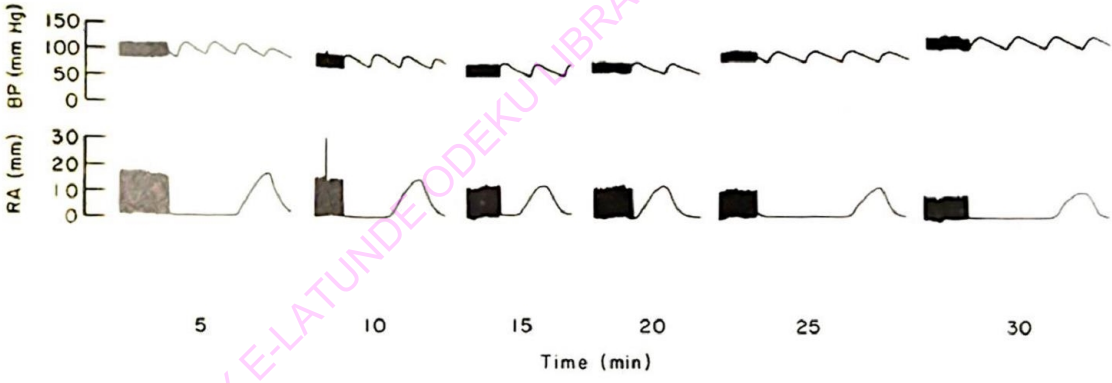

FIG. 2. A typical late BP and respiratory amplitude (RA) response to CUC. Note progressive fall of BP up to the 25th min.

Effects of CUC on sham-operated control

These were similar to those observed for group I for all the parameters measured.

0.15 M saline injections

Saline had no pharmacological effects in all the groups of animals studied.

Effects of simultaneous administration of CUC and 1 μg/kg adrenaline to adrenalectomized rats

Simultaneous injection of CUC and adrenaline reversed the increased susceptibility of adrenalectomized rats to CUC. The pattern of response was similar to that obtained in group I.

Discussion

The biphasic effect of CUC on BP observed

TABLE 2. Heart rate (per min) in groups I, II and III before and after CUC injection

	Post-injection period (min)								
	Control	5	10	15	20	25	30	50	70
Group I	360.4 ±	316.0* ± 10.1	318.7** ± 13.5	316.9** ± 16.0	321.1** ± 13.2	322.2** ± 14.6	327.3 ± 15.6	336.5 ± 13.4	344.9 ± 10.6
Group II	340.0 ± 9.6	288.0** ± 18.1	277.5* ± 16.8	285.5* ± 12.6	300.5** ± 11.7	311.0 ± 11.5	316.0 ± 12.7	342.0 ± 12.6	351.0 ± 10.8
Group III	343.1 ± 9.8	266.1* ± 23.3	248.8* ± 23.6	257.2* ± 25.7	253.3* ± 29.3	269.3** ± 31.5	304.2 ± 26.6	326.5 ± 29.5	356.0 ± 19.5

TABLE 3. Respiratory rate (per min) in groups I, II and III before and after CUC injection

	Post-injection period (min)										
	Control	5	10	15	20	25	30	50	60	65	70
Group I	58.8 ± 4.4	59.1 ± 8.1	64.3 ± 6.2	59.1 ± 5.0	64.3 ± 6.7	64.3 ± 4.1	68.5 ± 4.8	72.8* ± 4.6	74.6* ± 5.3	73.7* ± 4.2	72.8* ± 4.4
Group II	63.0 ± 3.1	55.8 ± 8.2	57.0 ± 8.0	60.0 ± 6.8	64.2 ± 5.4	66.6 ± 4.4	66.0 ± 4.4	70.8 ± 4.8	74.4 ± 5.6	76.8 ± 5.7	74.0 ± 5.9
Group III	54.2 ± 2.4	39.1 ± 7.5	36.7* ± 5.8	36.0* ± 4.9	40.0 ± 7.7	45.0 ± 8.1	51.4 ± 5.6	59.0 ± 5.8	48.0 ± 9.2	49.0 ± 11.0	57.6 ± 7.0

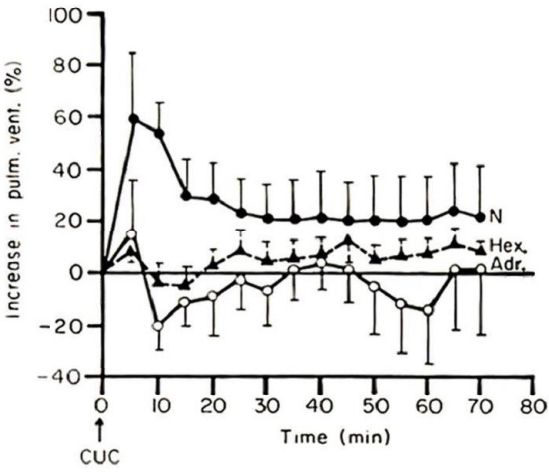

FIG. 3. Increase in pulmonary ventilation (IPVR) in normal (N), hexamethonium-treated (Hex.) and adrenalectomized (Adr.) rats after CUC injection.

in groups I and III in this study is consistent with earlier findings in normal dogs (Elegbe & Oyebola, 1977) and cats (Elegbe *et al.*, 1976). The absence of the initial transient hypotension in the hexamethonium-treated group is most probably due to blockade of the inhibitory effects of parasympathetic nerves to the heart by the ganglion blocker. Also, the higher early pressor effect observed in this group (Group II) is most probably due to the absence of inhibitory parasympathetic impulses to oppose the pressor action of the catecholamines released from the adrenal medulla. Nicotine, a component of CUC, has been shown to be capable of direct stimulation of the adrenal medulla leading to release of catecholamines (Armitage & Milton, 1965).

The ECG findings are consistent with earlier findings in cats (Elegbe *et al.*, 1976).

The significantly greater reduction in BP, HR and IPVR in group III compared with group I and the inability of group III animals to survive a second injection of CUC show that adrenalectomized animals have increased susceptibility to CUC-induced toxicity/lethality. Since the responses of the sham-operated animals were similar to those of group I, the different response pattern in group III cannot be attributed to the surgical exposure of the adrenal glands; rather it is due to the actual removal of the glands.

The hexamethonium-treated rats survived the toxic effects of CUC better than the adrenalectomized rats. However, in the latter group, simultaneous injection of CUC and adrenaline corrected the impaired resistance to CUC toxicity. These results show that catecholamines from the adrenal gland are more important than those from post-ganglionic adrenergic nerve endings in the cardio-respiratory response to CUC.

The results of this study showed for the first time that in death from CUC, the primary event is respiratory failure. This is followed by cardiovascular collapse. Respiratory arrest preceded the precipitous fall in blood pressure by roughly 1 min. The implications of this finding for the resuscitation of cases of CUC poisoning merits further studies. The finding, however, suggests that respiratory support deserves priority attention over signs of cardiovascular dysfunction such as hypotension and bradycardia in the management of the CUC poisoning.

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