Br. J. Pharmac. (1971), **41**, 302–316.

Effect of catecholamines and angiotensin on extracellular water and cation movements and the effect of α and β-adrenoceptor blockade

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Summary

1. The extracellular fluid volume (ECFV), as raffinose space, and its content of Na, K and Ca were measured in anaesthetized dogs in acute experiments before and during the vascular response to intravenous injections and infusions of noradrenaline, adrenaline, isoprenaline and angiotensin.

2. In male dogs the effect of noradrenaline was unpredictable, the ECFV might increase or decrease. In female dogs noradrenaline caused an increase in the ECFV. The difference between the responses of the two sexes was statistically significant ($P<0{\cdot}001$). After phentolamine, noradrenaline exerted no effect at all in either sex. After bretylium the results were like those in normal animals.

3. In both males and females adrenaline generally induced an increase in the ECFV. After phentolamine, adrenaline decreased the ECFV in males and caused little change in females. The differences before and after blockade were statistically significant ($P<0{\cdot}001$). After bretylium the results in both sexes were like those in normal animals.

4. In both male and female dogs isoprenaline induced an increase in the ECFV and the results were the same as in the normal animals after both phentolamine and bretylium.

5. In male dogs there was no change in the ECFV as a result of administering angiotensin, either alone or in the presence of phentolamine or bretylium. In normal females angiotensin induced a decrease in ECFV and the difference between the responses of the males and females was statistically significant ($P<0{\cdot}005$). In females which had received either phentolamine or bretylium the results were indistinguishable from those in the males.

6. Blockade of the β-adrenoceptors with pronethalol in a few animals did not change the response to the drugs from those seen in normal animals.

7. The cation content of the ECF changed in the same direction and to about the same extent as the water, except after noradrenaline when in some experiments the proportionate change in potassium concentration was considerably greater than that of the other substances.

8. The inulin space and its Na and K content were measured in several dioestrous, oestrous and pro-oestrous rats and in normal and stilboestrol treated

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males before and after giving an intravenous injection of angiotensin. There was little difference between the results of control injections of 0·9% saline solution and of angiotensin in dioestrous and oestrous females and normal males. On the other hand, pro-oestrous females and stilboestrol treated males responded alike to angiotensin in the form of a decrease in ECFV which was statistically different from the responses in the other three groups ($P<0·0005$).

9. It is suggested that the various results depend on two factors: the site of action of the drug—for example, whether it increases or decreases capillary pressure and therefore, fluid transfer—and also the sex of the animal. The ground substance of the small blood vessels is probably important in taking up and releasing fluid, and its capacity for so doing may well vary with the amount of available oestrogen. It appears that the effect of oestrogens and events at the α-adrenoceptor site are connected in some way.

Introduction

In the course of a series of experiments on the mode of action of oxytocin it was considered necessary to examine the volume changes of extracellular fluid that occurred acutely when this hormone was given intravenously to anaesthetized dogs and to compare the results with those found when other vasoactive substances were given. The results with these other substances were unexpected and the following is an account of the findings in dogs when noradrenaline, adrenaline, isoprenaline and angiotensin were used and in rats given angiotensin. In addition to the changes in volume of the extracellular fluid estimated as inulin or raffinose space, the content of Na, K and Ca in raffinose space was measured.

What was surprising was the complete lack of relationship between the direction of the blood pressure and volume changes and also the clear indication of a sex difference in the response to the pressor vasoactive substances. A preliminary account of this work has already appeared (Pickford, 1969).

Method

Rats and young adult hybrid dogs were anaesthetized with sodium pentobarbitone (50 mg/kg and 26·5 mg/kg body weight, respectively). For the dogs the renal pedicles were firmly ligated through a midline abdominal incision and a known quantity of raffinose dissolved in 0·9% NaCl solution was injected intravenously. Cannulae were tied into the divided ends of one femoral vein and joined by a piece of rubber tubing forming a short external venous path into which it was easy to give injections and from which blood samples could be collected quickly by means of clean dry needles and syringes. Each blood sample collected was just over 2 ml. Before blood was allowed to flow through the short external path, heparin (5 mg/kg body weight) was injected intravenously. Blood pressure was recorded on smoked paper by means of a mercury manometer (1 mmHg$\equiv$1·333 mbar) connected to a cannula inserted either into a carotid artery or the femoral artery of the leg opposite to that from which blood was collected. Particular care was taken to tie off all bleeding points, however small. No observations were made until at least 1 h after the raffinose had been given. The number of observations made in any one dog varied according to its size and whether blood loss was satisfactorily controlled. When single injections were given a control blood sample was collected immediately

before the injection and a second sample was taken when the change in blood pressure reached its maximum, which was in 0·75–1·0 minutes. When intravenous infusions were used they were given by means of a Palmer constant infusion pump at 0·2–0·4 ml/minute. A control sample of blood was taken and the infusion begun at once. Three samples were collected during the infusion which lasted for 5 minutes. The blood pressure stabilized between the first and second samples, that is, in 1·0–1·5 minutes.

When rats were used the renal pedicles were firmly ligated through a midline abdominal incision which was then closed and 1·5–1·7 mg inulin in a 10% w/v solution injected through a fine polythene cannula inserted into either a femoral or external jugular vein. Inulin was used rather than raffinose since the amount necessary could be injected in small volume (0·15–0·17 ml). Not less than 1 h after this a carotid artery was cannulated with fine polythene tubing, a blood sample collected of slightly less than 1 ml and, at once, the drug under investigation injected intravenously in a volume of 0·1 ml. A second carotid blood sample was collected 0·75–1·0 min later.

Inulin and raffinose concentrations were estimated by a slight modification of the method of Higashi & Peters (1950) for inulin, so that only 0·2 ml plasma was needed. The amount of raffinose given was three times the amount of inulin that would have been needed since, on incubation with acid, raffinose produces three times less fructose.

A flame spectrophotometer (Unicam SP 900) was used for estimation of Na, K and Ca.

Drugs were dissolved in 0·9% NaCl solution and the volume of single injections given to dogs was never greater than 1 ml. The drugs used were: noradrenaline acid tartrate (Levophed, Bayer), adrenaline chloride (Adrenalin, Parke Davis & Co.), angiotensin II-asp-β-amide (Hypertensin, Ciba), phentolamine mesylate (Rogitine, Ciba), bretylium tosylate (Burroughs Wellcome & Co.), pronethalol (Alderlin, I.C.I.), propranolol (Inderal, I.C.I.), stilboestrol diproprionate (Burroughs Wellcome & Co.) and inulin (Kerfoot). The least doses of the blocking agents used were as follows: phentolamine, 0·44–0·5 mg/kg; bretylium, 20–25 mg/kg; pronethalol, 4–5 mg/kg; and propranolol, 3–3·5 mg/kg. The doses of the vasoactive substances are mentioned in the text. All doses are expressed in terms of the salts.

Results

In those experiments in which bleeding was clearly well controlled the extracellular fluid volume (ECFV) did not change by more than 100–150 ml over a period of 1·5–2·25 hours. When bleeding was poorly controlled the change was considerably more than this. When bleeding persisted experiments were ended in about 1 hour. However, even in these unsatisfactory experiments results fell in the normal range when expressed as a percentage change, possibly because control and experimental samples were collected over a short interval of time.

Noradrenaline

Effects of single intravenous injections In three males the injection was repeated in the same dose after an interval of 15–20 min; the pairs of results were similar to within 5%. Figure 1 on the left shows the effect of noradrenaline (10–20 μg) on

the extracellular fluid volume (ECFV) and its cation content in sixteen male dogs ; in two additional animals only the ECFV was measured.

All cation movements closely followed that of water except in four animals when K increased 2–8 times more than water.

The pressure changes induced varied from 12 to 40 mmHg. As can be seen, the direction of the volume changes varied over a wide range and bore no relationship to the degree of blood pressure change. In four dogs, following a dose of phentolamine which fully blocked the pressor response to noradrenaline, there was no significant change in the ECFV and its cations, that is, the changes observed before blockade were related to the vasoconstrictor activity of noradrenaline and not to some other property. Following the control observations with noradrenaline six dogs were given bretylium and in two of these only the ECFV was measured. Twenty to thirty minutes later and when the blood pressure was almost the same as initially, the injection of noradrenaline was repeated. In these circumstances the blood pressure rose by 40–50 mmHg. Figure 1 on the left (open circles) shows that water and cation movements fell within the same range as in the absence of the blocking agent, so there was still a varied response.

Attempts were made in the absence of phentolamine to block β-adrenoceptors completely as judged by the absence of any depressor action of isoprenaline. This was difficult to achieve. Pronethalol was more satisfactory than propranolol and appeared to induce block in three out of five males. Following this noradrenaline induced no change in ECFV in two dogs and an increase of 13% in one ; that is, the results fell in the normal range.

Fourteen female dogs were subjected to the same procedures and doses as the males and the results are shown to the right of Fig. 1. In three animals the ECFV only was measured. In two animals consecutive control injections were given and the paired results were similar. The blood pressure rose by 4–40 mmHg when noradrenaline was given. In all instances the ECFV either increased or was little affected. The cations moved in the same direction and to approximately the same extent as the water except that on six out of eleven occasions the total extracellular K increased 2–5 times more than the water. The difference between the responses of the males and the females is statistically significant by student's t test ($P<0{\cdot}001$). One of the females was well advanced in pregnancy and the shifts seen were the greatest increases plotted. Four females, including the pregnant animal, were given phentolamine in fully blocking doses and after this noradrenaline had no effect on the ECFV and its cations. Five females received bretylium, after which the shifts

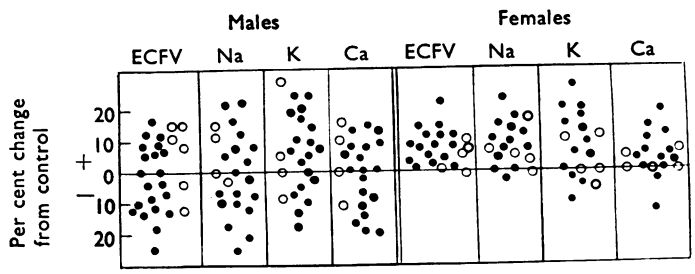

FIG. 1. Dogs. Percentage change from control of ECFV, Na, K and Ca on intravenous injection of noradrenaline in males (on left) and females (on right). ●, Normal animals ; ○, after bretylium.

occurring with noradrenaline were similar to those seen in unblocked animals (Fig. 1 on right, open circles). Blood pressure changes on giving noradrenaline after bretylium were from 28–44 mmHg.

Pronethalol without phentolamine was given to four females; in only one was the response to isoprenaline wholly suppressed. Before blockade noradrenaline caused a 10% increase in ECFV and after blockade no change.

Effects of infusions

Three males were given infusions of noradrenaline (20 μg/min) and the results are shown in the upper part of Fig. 2. The pattern of response was variable, as was that to single injections. Two females received infusions of 15 and 20 μg/min noradrenaline. The results are shown in the lower part of Fig. 2. There was no indication of a reduction in ECFV. This is in agreement with the findings from single injections.

Adrenaline

Effect of single injections

The observations were made in the same way as for noradrenaline. Fourteen males were used and consecutive injections of the same dose were given to two of these; the pairs of results were closely alike. In two additional animals the ECFV only was measured. The upper part of Fig. 3 on the left, shows that following adrenaline (15–25 μg) either the ECFV rose or did not change appreciably, except in one instance when it decreased. The cation shifts were in general similar to those of water. Blood pressure rose by 18–47 mmHg. Ten dogs, including the two in which only the ECFV was measured, were given phentolamine and in four of these there was not much alteration in the ECFV. The blood pressure changes induced by adrenaline in these four were as follows: falls of 14 mm, and 30 mm, a rise of 4 mm and no change. Thus, as with noradrenaline there was no correlation between the change in ECFV and pressure. In one of the remaining dogs there was some increase in ECFV and in the other five it decreased. The results after phentolamine differed significantly from those before ($P<0{\cdot}001$). Five dogs were given bretylium and in one of these the ECFV only was measured. After this blocking agent the results of adrenaline administration fell in the same range as in the unblocked dogs (Fig. 3).

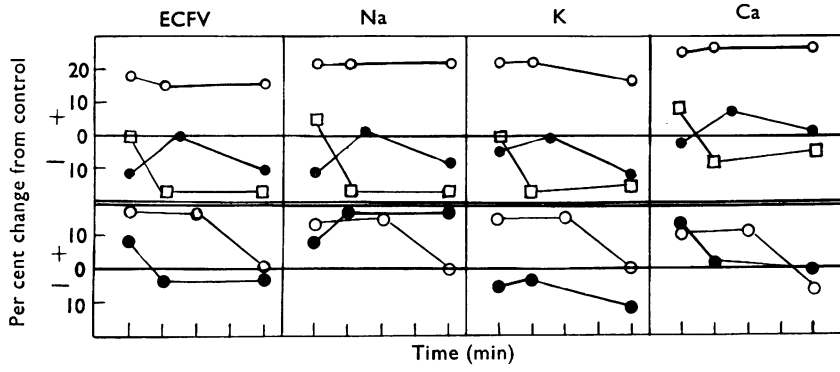

FIG. 2. Percentage change from control in ECFV, Na, K and Ca, in five dogs on intravenous infusion of noradrenaline. Above, males; below, females.

In two males, after β-adrenoceptor blockade with pronethalol in the absence of phentolamine, adrenaline caused no change in the ECFV and its cations.

Twelve females were used and two of them were given consecutive doses of adrenaline ; the paired results were similar. In two additional animals the ECFV only was measured. The lower part of Fig. 3 on the left shows that on giving adrenaline (15–25 μg) there was usually an increase in the ECFV and its cations. The blood pressure was raised by 15–50 mmHg.

Phentolamine was given to nine dogs (lower part of Fig. 3 on right), after which there was no instance of an increase in ECFV, but rather a tendency to a small decrease in both it and its cation content. In three of these dogs the ECFV only was measured. There was a significant difference between the effects of adrenaline in the normal and in the phentolamine blocked animals ($P<0{\cdot}001$). Two of the dogs used were in late pregnancy. When unblocked one of these gave the highest values plotted on the left of Fig. 3 and those from the other fell in the middle of the range. The former animal was given phentolamine and its responses were then indistinguishable from those of the nonpregnant animals. Bretylium was given to four animals, one of which was the second pregnant dog. No difference was apparent between the results before and after bretylium block (Fig. 3, lower right).

In the absence of phentolamine and following successful β-adrenoceptor blockade with pronethalol in one animal, adrenaline caused a 9% decrease in ECFV.

Effects of infusions

Three males received infusions of adrenaline (20 μg/min). In two of them the ECFV increased and in one it decreased in the 5 min sample (Fig. 4, upper part). Two females were given infusions (10 μg and 15 μg/min). The initial effects differed but by 5 min both were alike and showed a small increase in ECFV (Fig. 4, lower part).

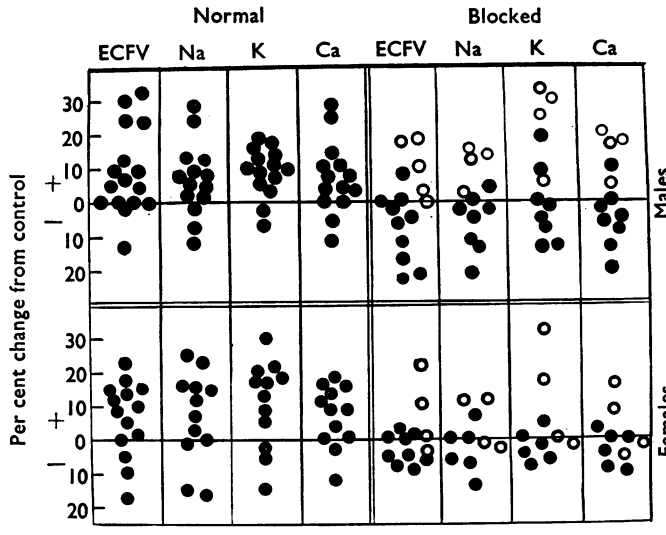

FIG. 3. Dogs. Percentage change from control of ECFV, Na, K and Ca on intravenous injection of adrenaline in males (above) and females (below). On left, normal animals ; on right, ●, after phentolamine, ○, after bretylium.

Isoprenaline

Effects of single injections

Sixteen male dogs were used in three of which the ECFV only was measured. With the doses given (5–12·5 μg) the pressure fell by 17–45 mmHg. In most instances the ECFV and total extracellular cations rose; any decrease seen was moderate (Fig. 5, upper part on left). Doses of phentolamine which fully blocked the response to noradrenaline were given to ten dogs and bretylium to five. After phentolamine the results were widely scattered and after bretylium were near the zero line (Fig. 5, upper part on right). There was, however, no statistically significant difference between the results from the normal and the blocked dogs.

Eleven female dogs were used and the results were similar to those seen in the males (Fig. 5, lower part on left), namely in most instances an increase in ECFV.

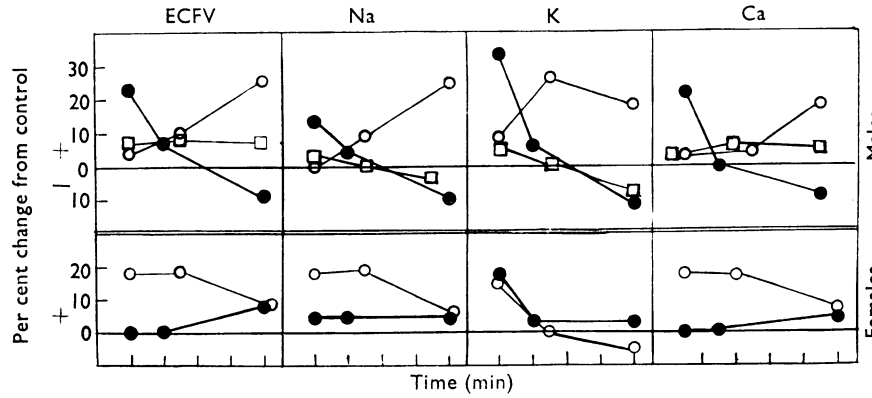

FIG. 4. Percentage change from control in ECFV, Na, K and Ca in five dogs on intravenous infusion of adrenaline. Above, males; below, females.

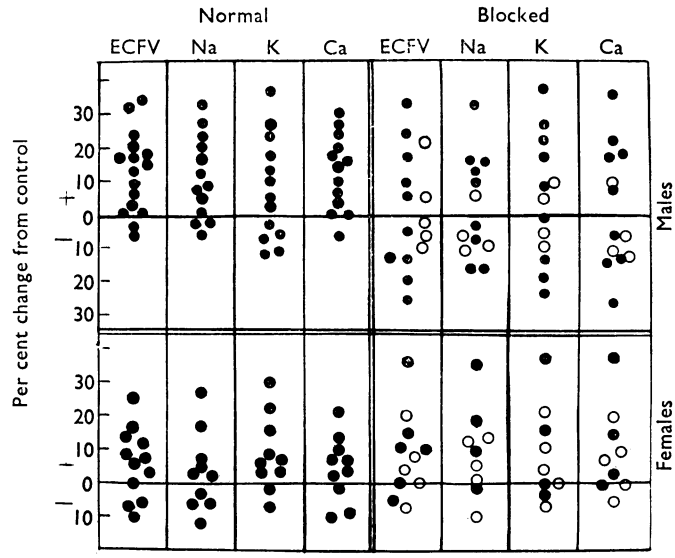

FIG. 5. Dogs. Percentage change from control in ECFV, Na, K and Ca on intravenous injection of isoprenaline in males (above) and females (below). Left, normal animals; right, ●, after phentolamine, ○, after bretylium.

One female was given two consecutive control doses of isoprenaline and the results were closely similar. Six of the females were given phentolamine and five bretylium. These agents did not induce any change from the normal pattern of water and cation movement (Fig. 5, lower right). One of the females was well advanced in pregnancy and both before and after bretylium the results fell within the range of values found in the non-pregnant animals. In no circumstances was there a statistically significant difference between the responses of the males and females.

In three males and one female pronethalol successfully blocked the β-adrenoceptors and thereafter in all four, isoprenaline caused no change in the ECFV or its cations.

Effects of infusions

In the upper part of Fig. 6 it can be seen that infusions of isoprenaline (10–20 μg/min) into males caused a maintained increase in the ECFV. The lower part of Fig. 6 shows that females given similar infusions also responded by an increase in ECFV. That is, in both sexes the infusions led to the same result as the single injections.

Angiotensin

Effects of single injections

The upper part of Fig. 7 on the left, shows that in twelve males doses of angiotensin (5–7·5 μg) that raised blood pressure by 14–52 mmHg had negligible effects on the ECFV or its cation content. In three of them the same dose was given twice to each and the pairs of results did not differ by more than 4%. On the right of Fig. 7 (upper part) it is shown that after fully blocking doses of phentolamine the results in eight dogs were not significantly different from those before blockade. Two of these dogs each received two injections of angiotensin. The phentolamine caused a fall in blood pressure which varied from 15 to 33 mmHg and after it angiotensin raised the pressure by 25–52 mmHg. Four dogs were given bretylium and in its presence the effect of angiotensin was only slightly more variable than before blockade. One of the bretylium treated dogs received angiotensin twice and the other three once each. Following bretylium angiotensin raised the blood pressure by 10–15 mmHg more than in the untreated dogs.

The lower part of Fig. 7 shows the results for eighteen female dogs in four of which the ECFV only was measured. On the left are the results when angiotensin was given to normal dogs in doses (5–7·5 μg) that raised blood pressure by 12–50

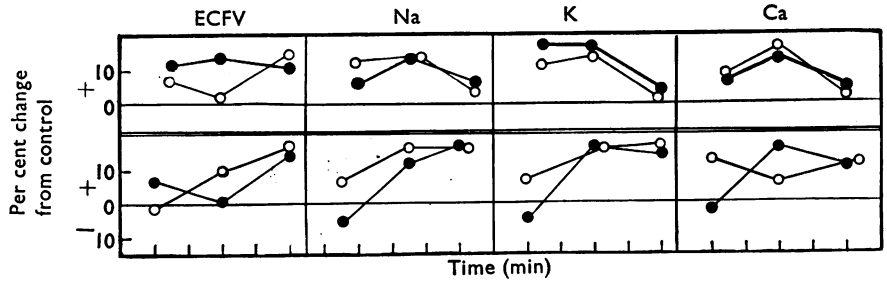

FIG. 6. Percentage change from control in ECFV, Na, K and Ca in four dogs on intravenous infusion of isoprenaline. Above, males; below, females.

mmHg. In two animals the control injections were repeated and the paired results were similar. It can be seen that the ECFV either did not change or it decreased, sometimes considerably. The cations moved in the same direction and to approximately the same extent as the water. The difference between the results found in males and females is statistically significant ($P<0{\cdot}005$). On the right of the lower part of Fig. 7 are shown the results in females after fully blocking doses of phentolamine which reduced blood pressure by 12–28 mmHg. When angiotensin was given after blockade the ECFV either changed very little or increased. The cation shifts were slightly more variable. There was a significant difference in the water movement of females before and after phentolamine blockade ($P<0{\cdot}001$). Following blockade there was no difference between the male and female responses. Four females received bretylium and the results fell in the same range as after phentolamine.

In three males and three females following the α-adrenoceptor blockade pronethalol was given and the injections of angiotensin repeated. The results were entirely similar to those with α-adrenoceptor blockade alone. In the three males and one female in which pronethalol in the absence of phentolamine successfully blocked the response to isoprenaline, the ECFV decreased twice (12% and 12%) and twice showed no change.

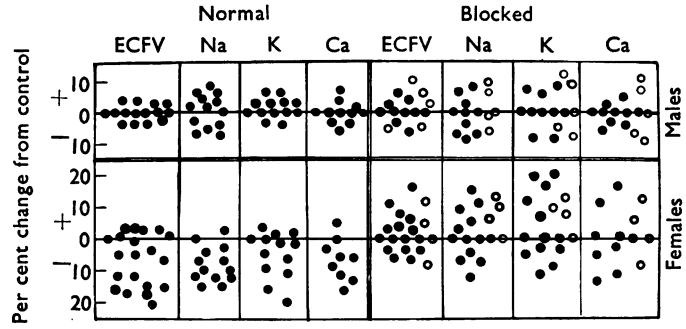

FIG. 7. Dogs. Percentage change from control in ECFV, Na, K and Ca on intravenous injection of angiotensin in males (above) and females (below). Left, normal animals; right, ●, after phentolamine, ○, after bretylium.

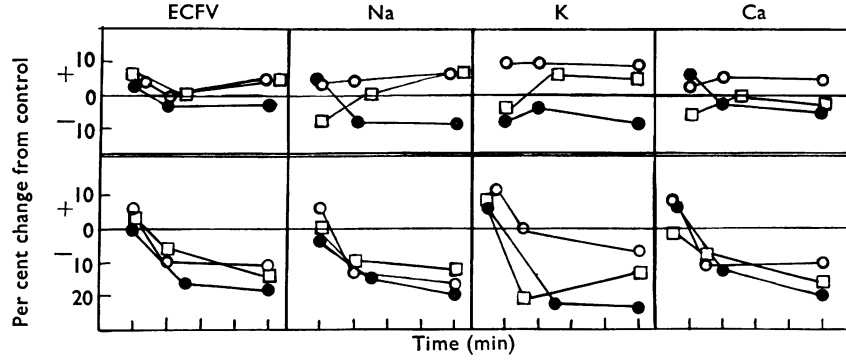

FIG. 8. Percentage change from control in ECFV, Na, K and Ca in six dogs on intravenous infusion of angiotensin. Above, males; below, females.

Effect of infusions

Three males were given infusions of angiotensin (2–5 μg/min). In the upper part of Fig. 8, it can be seen that angiotensin made little difference to the ECFV and its content of cations.

Three females also received similar infusions and in these the ECFV and its total cation content decreased (Fig. 8, lower part).

Rats

It seemed as well to find out whether a sex difference in the water shift response to angiotensin could be demonstrated in a species other than the dog and at the same time to test the working hypothesis that oestrogens were the agents responsible for the sex difference. Male and female rats were used similarly to dogs except that only one observation could be made on each animal. The inulin space was measured in twenty dioestrous, nineteen oestrous, and fourteen pro-oestrous females. Thirty normal males were studied and twenty-two to which stilboestrol (25–30 μg) had been given subcutaneously the day before observation. All the animals were between 2·5 and 4·5 months of age. In addition control observations were made in ten males and eleven females in which 0·9% NaCl solution was given instead of angiotensin between the collection of the two blood samples ; the second sample was always taken 1 min after the intravenous injection which was in a volume of 0·1 ml. When angiotensin was used the dose was 8–10 ng. The ECFV in the controls did not vary from the first to second sample by more than 7%. In the normal males given angiotensin the change in ECFV was scarcely greater than in the controls and suggests that in fact, the shift of ECFV was negligible. The change induced in dioestrous and oestrous females was rather more variable than in the males but there was no significant difference between these three groups. On the other hand pro-oestrous females reacted in a manner that was significantly different from the other groups ($P<0·0005$) and also the stilboestrol treated males as compared with the normal males ($P<0·002$), (Fig. 9). As well as inulin, plasma Na and K were estimated in fourteen normal males and in four dioestrous and three oestrous females. As far as could be seen in these few animals Na and K shifted to about the same extent as water, that is, very little.

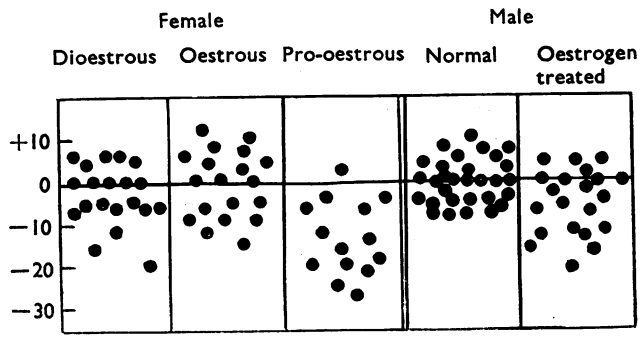

FIG. 9. Rats. Percentage change from control in ECFV on intravenous injection of angiotensin to dioestrous, oestrous and pro-oestrous females and normal and stilboestrol treated males. Males given 25–30 μg stilboestrol subcutaneously the day before observation.

Discussion

Raffinose was used as the marker for the extracellular space in dogs because its molecule is smaller than that of inulin and more uniform in size, and it was felt that it would distribute more rapidly and move faster when extracellular exchanges occurred and, perhaps, therefore give more accurate information. The technique of single injections was preferred to that of infusions because only two blood samples were needed with the former as against four with the latter ; that is, more observations could be made with less exsanguination. It is believed that the single injection method supplied valid information since the results were similar to those of the slower infusion method. The many single injections gave a greater scatter of responses than the fewer infusions, possibly because a single injection is a more violent assault than an infusion ; the drug concentration builds up rapidly and there is less time for adaptive mechanisms to smooth out the response.

A search of the literature has revealed a considerable volume of work on the effects of haemorrhage and nerve reflexes on ECFV, but very little on the acute effects of drugs and hormones. The present work gives information only about the total ECFV measured as raffinose space. At the time the experiments were done no means were to hand for estimating the plasma volume independently of the raffinose space, except by the use of a dye such as Evans blue and this would have made simultaneous estimations of raffinose impossible. Work using iodinated albumin has begun in order to see whether plasma and interstitial space share equally in the adjustments that occur when the blood pressure is altered by means of drugs. The total ECFV changed rapidly, as much as a 30% change occurring in some instances in a period of 1 minute. Oberg (1964) noticed that a substantial amount of fluid could be transferred across the capillary membranes in a fairly short period of time.

The question is, where does the fluid come from and where does it go? There are various tissues that could take it up or release it. Friedman & Friedman (1961) suggest on the evidence of a number of experiments in which drugs were used on whole animals and isolated arteries, that fluid moves into and out of the active smooth muscle cells of the blood vessels. They related contraction with movements of Na and water into cells and relaxation with an outward movement. They found that Na and water did not necessarily move to the same extent, particularly when noradrenaline was given and that, on occasion K moved in a direction opposite to that of Na, though this was never seen after angiotensin. They also concluded that the extent of the shift of water was unrelated to the order of blood pressure change. Thus, the present results are partly in agreement with those of Friedman & Friedman (1961), for example, in the female when angiotensin was given and in normal males and females when adrenaline or isoprenaline was given ; they are also similar in that blood pressure and extent of fluid shift showed no relationship to each other.

However, there are differences between the present results and those of Friedman and Friedman, as well as additional observations which cannot be explained by their thesis alone. Thus, there is the fact that water shifts were very different when the pressure was raised with noradrenaline as compared with angiotensin and that the direction of the shift can change even when the pressure change is the same as before. Smooth muscle cells may take up and release some part of the water and cations, but since changes in capillary pressure and hence transfer, must take place,

extravascular tissue is probably also involved. In all likelihood this will be connective tissue, especially that of the ground substance of the blood vessels. As far as particular body regions are concerned in the process, Oberg (1964) found that those containing muscle and skin took a large part in adjustments of fluid transfer in response to various cardiovascular reflexes and that transfer across the capillaries of the intestines was unimportant. Another possibility is that, when fluid enters the extravascular space, the lymphatics may carry away some of it. According to Wegelius & Asboe-Hansen (1956) and Chvapli (1967) mast cells, which tend to accumulate around the small blood vessels, can share in water binding by means of liberation and depolymerization of their granules. Further reference to connective tissues will be made later.

It is not surprising that the mean blood pressure bears no direct relationship to the changes in ECFV since the former will be determined by the cardiac output as well as by the state of the arterioles, venules and the micro-circulation. The state of tension in the small vessels is a factor that has to be considered because it could modify their permeability, especially perhaps, after treatment with phentolamine. However, if the tension of the vessels is important the results with angiotensin, for instance, show that it cannot be the only significant factor involved.

In general the results were as follows: the two constrictors exerted different effects ; there was a difference in the response to adrenaline but not isoprenaline after phentolamine ; there was a striking sex difference between the responses of normal males and females to angiotensin ; noradrenaline had very variable effects in males and fairly consistent ones in females. There are, then, two problems to be considered relative to the changes in ECFV ; one is the site and mode of action of the drugs and the other is the role of the sex of the animal. If for the time being the sex differences are ignored and the site of action considered it is perhaps not surprising that there should be dissimilar movements of water with the two vasoconstrictors, noradrenaline and angiotensin. The former easily constricts most vessels (Haddy, Fleischman & Emanuel, 1957), including the small veins, whereas angiotensin preferentially constricts arterioles. With the doses of the latter used here probably most of the arterioles would constrict, not merely those of the more sensitive regions.

With these two agents therefore, the total mass of contracting smooth muscle would be different and capillary pressure changes dissimilar, as would also capillary transfer of fluid and electrolytes. Adrenaline and isoprenaline may exert similar effects for different reasons. The action of adrenaline is complicated by its double role as constrictor and dilator. Eckstein & Abboud (1967) found that in the forearm of man isoprenaline induced a marked arteriolar dilatation without much change in venous tone and that adrenaline given intra-arterially exerted a venoconstrictor effect only. Somewhat similar results have been seen in dog (Haddy *et al.*, 1957). Thus, both substances would, for different reasons, raise capillary pressure and this could be why they induce similar results as far as water shift is concerned.

Table 1 summarizes the findings on ECFV after giving the blocking agents which affect the sympathetic constrictor system. After bretylium the responses to noradrenaline were the same as in the normal animal and after phentolamine they were wholly eliminated ; that is, whatever occurred depended on the constrictor activity of noradrenaline. Likewise the results following isoprenaline depended on its depressor action since it exerted no effect at all in the four dogs in which β-adreno-

ceptor blockade was satisfactorily achieved. Bretylium did not change the response to adrenaline, whereas phentolamine changed it in both sexes. Thus with both noradrenaline and adrenaline an intact α-adrenoceptor area was necessary for normal responses of water shift. When this was blocked adrenaline exerted little, if any, effect in the female ; the response of the male, too, was changed and in some instances was even reversed. Neither phentolamine nor bretylium affected the male response to angiotensin, whereas both affected that of the female. These last observations mean that any link between the effect of angiotensin and release, reception and reuptake of noradrenaline cannot alone account for the findings—the sex of the animal is also important.

It is not difficult to see why phentolamine should differentiate between isoprenaline and adrenaline ; the action of the former will be unchanged by the blockade and the venoconstrictor action of the latter will be eliminated. But it is not easy to understand why the unmasked dilator activity of adrenaline does not show itself in the same way as that of isoprenaline. The remaining observed effects of the blocking agents seem to be bound with the sex of the animal and will therefore be considered below.

An earlier observation on rats (Lloyd & Pickford, 1967a) showed that in animals given ganglion and peripheral sympathetic blocking agents there was little, if any, change from the normal inulin space volume of males and a considerable change in females ; that is, a sex difference was apparent. There is some indication from previous work and from the present experiments that there may be a tie between the α-adrenoceptors and sex steroids. On the other hand, such information as is available on the effects of β-adrenoceptor blockade alone or in combination with α-adrenoceptor blockade, suggests that reactions arising from the noninnervated β site are not affected by the sex of the animal.

Previous work relating oestrogens and the sympathetic system is as follows. In the normal conscious or anaesthetized dog, and in several other species including man, oxytocin dilates a number of vascular beds. However, it acts as a constrictor agent in the same beds following administration of an oestrogen or after removal of sympathetic activity either by chemical or surgical means (Lloyd & Pickford, 1967b). Oestrogens affect cell permeability in general and the capacity of tissues to take up water (Asboe-Hansen, 1966 for refs.) ; in particular the ground substance around the smaller blood vessels is affected. For example, Bonta, Vos & Delver (1965) showed that in the heart-lung preparation and within 15 min of its administration, oestriol 16–17 disodium succinate delayed the appearance and diminished the intensity of the haemorrhages induced by the application of Naja-Naja and cobra venom to the surface of the lung. They suggested that the results could be accounted for by the ability of oestrogens to promote polymerization of tissue glycopeptides, particularly those of the ground substance of the blood vessels, so that

TABLE 1. *Effect of blocking agents on drug induced changes in extracellular fluid volume*

	Phentolamine		Bretylium	
	Male	Female	Male	Female
Noradrenaline	−ve	−ve	0	0
Adrenaline	+ve	+ve	0	0
Isoprenaline	0	0	0	0
Angiotensin	0	+ve	0	+ve

−ve No response.
0 Response as in the unblocked state.
+ve A change of response from that in the unblocked state.

the strength of the vessels walls was enhanced. The rapidity of the effect was also noted by Schiff & Burn (1961a) who found an increase in metachromasia, that is in the size of the glycopeptides, within 15 min of injection of a conjugated oestrogen. This change was particularly noticeable in the area around the small blood vessels. Schiff & Burn (1961b) also noted that oestrogens increased the number of mast cells around the blood vessels and limited their degranulation and that the increase in number of these cells was greater in females than in males. As long ago as 1935 Zuckerman described the swelling of the sexual skin in monkeys and the role of oestrogens and glycopeptides in this phenomenon. There is therefore ample evidence that oestrogens can modify the quantity and nature of the glycopeptides and that these substances have an important water and electrolyte retaining property (Asboe-Hansen, 1966). There is little evidence implicating androgens.

The other part of the present problem is the relationship between the sympathetic nervous system and oestrogens. It has been appreciated for a long time that oestrogens promote the vascularity of many tissues and not only those of female reproductive organs. Since oestrogens spare mast cell degranulation it seems unlikely that the heightened vascularity is caused by this source of histamine (Schiff & Burn, 1961b). Reference has already been made to some recent work which showed that in both males and females elimination of sympathetic activity induced a vasoconstrictor response to oxytocin as readily as administration of oestrogens (Haigh, Lloyd & Pickford, 1965). It was further observed that after surgical unilateral lumbar sympathectomy and until such time as the nerves had degenerated, the dilator response to oxytocin on that side could be restored either by stimulation of the peripheral part of the divided nerve or by an infusion of adrenaline in low concentration. Noradrenaline was ineffective.

It was suggested that oestrogens interfered in some way with a catecholamine, possibly adrenaline itself, which accompanied or developed from the sympathetic neurotransmitter when it was released. The importance of adrenaline may lie in its metabolic activity. Further, the earlier work showed that if the α site was blocked adrenaline was no longer able to restore the dilator effect of oxytocin ; block of the β site was immaterial. This older work appears to have relevance to the results of the present experiments. Thus, there is a clear cut sex difference in the water shift when the pressure is raised with angiotensin and it disappears following α blockade, when the female response became like that of the male. Angiotensin was the only substance used whose effect on water shift was changed, in the female, in the presence of bretylium ; with none of the other agents was bretylium observed to affect the result. No explanation can be offered. It may be mentioned here that experiments now almost complete show the existence of a marked sex difference in water movement when oxytocin is given and that after block of the α site the response of the female here, too, becomes like that of the male.

It is not possible to say whether there is a direct connexion between the α receptor site and the effect of oestrogens or whether α site blockade and oestrogens lead to the same result for different reasons. But it does appear that events at the α site have importance relative to the movement of ECF. The work on rats supports the hypothesis that oestrogens are concerned with the changes observed since pro-oestrus is the time of maximum surge of oestrogen (Exley, 1971), and the stilboestrol treated males reacted like pro-oestrus females. Further, this work shows that at least two species behave similarly.

This discussion has been concerned with some of the factors that may be involved in the observed effects of the drugs used. At present it is impossible to propose any hypothesis that will cover all the findings, beyond saying that the site of action of the drug is a factor, that α-adrenoceptor activity seems to be in part sex dependent and that, from their known actions, it is probably oestrogens and not androgens which are the responsible agents. So far one point has not been mentioned, namely the possibility that raffinose gives an over-estimation of the fluid shift. Even if this is the case, the sex difference remains to be explained. Further work needed includes finding out what happens to the ECFV when oestrogens or testosterone are given in conjunction with the vasoactive substances to normal and gonadectomized animals.

Our thanks are due to the Royal Society for a grant to purchase an SP 900 flame photometer and to Imperial Chemical Industries for gifts of Alderlin and Inderal. We wish to thank Mr. James Robertson for making the cation estimations.

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(*Received August* 24, 1970)