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THE EFFECT OF A XANTHINE DERIVATIVE, 1-(5'OXOHEXYL)-3-METHYL-7-PROPYLXANTHINE (HWA 285), ON HEART PERFORMANCE AND REGIONAL BLOOD FLOW IN DOGS AND RABBITS

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1 The effect of a new xanthine derivate 1-5' oxohexyl-3-methyl-7-proplyxanthine (HWA 285) was studied on heart performance in dogs and rabbits and on regional blood flow in rabbits.

2 Heart performance (cardiac output and dP/dt *max*) in dogs was increased. Cardiac work (calculated as CO × mean BP) was not changed in dogs and did not change or was slightly decreased in rabbits. Heart rate was increased in dogs and unchanged in rabbits.

3 Blood pressure decreased slightly in dogs, and more markedly in rabbits. Total peripheral resistance was decreased in both species.

4 Regional blood flow (studied by use of 15 μm labelled microspheres) was increased in the heart, brain and skeletal muscle; the increase was dose-dependent in the range 0.3, 1.0 and 3.0 mg HWA 285 per kg intravenously. The highest dose produced a 2 fold decrease in the peripheral resistance in the brain, a 2.5 fold decrease in the heart and 4 fold decrease in skeletal muscle.

5 The drugs preferentially dilated small (7 to 10 μm) rather than larger (12 to 17 μm) arterioles; 9 μm microspheres were found in the outflowing blood after application of the drug, and the calculated blood flow increases were smaller, or absent, as compared with values obtained with 15 μm microspheres.

Introduction

It has long been known that different xanthines have a positive chronotropic and inotropic effect (e.g. Hedbom, 1899; Walton & Brodie, 1947; Nayler, 1963; Nayler, Rosenbaum, McInnes & Lowe, 1966; Kukovetz & Poch, 1969) as well as a dilator effect in coronary vessels (e.g. Smith, Miller & Graber, 1925; Essex, Wegria, Herrick & Mann, 1940; Michaelis, 1941; Boyer & Green, 1941). With the synthesis of xanthine derivatives having increased solubility in water, the clinical use became more important as they were shown to produce vasodilatation in different vascular beds (Popendiker, Boksay & Bollmann, 1971).

The mechanism of action of different xanthine derivatives as well as their effect seems to be related to the substitution in different positions: e.g. theophylline (1,3-dimethyl-xanthine) or any other xanthine derivative with a substituent in 1-position blocks the action of adenosine, probably by competing for its receptors (Scholtholt, Nitz & Schraven, 1972) and produces slight bradycardia (Sakurai & Komarek, 1974), whereas pentoxifilline [(3,7-dimethyl-1-(5-oxohexyl)-xanthine] blocks phosphodiesterase activity (Stefanovich, 1973) and thus increases the content of cyclic adenosine 3',5'-monophosphate (cyclic AMP) (Triner, Vulliemoz, Schwartz & Nahas, 1970) with a positive chronotropic and inotropic effect on the heart (Sakurai & Komarek, 1974) and a dilator effect in different vessels in the periphery (Popendiker *et al.*, 1971). Exchange of the methyl group of pentoxifilline in position 7 for a propyl residue increases its solubility in lipids and is supposed to improve its transport across the capillary wall and into the cells. It was therefore of interest to study the effect of this compound, 1-(5' oxohexyl)-3-methyl-7-propylxanthine (HWA 285, synthesized by Hochst, A. G., Werk Albert, Wiesbaden) on both cardiac performance and regional blood flow. Since the chronotropic effect may vary with the initial heart rate, the studies were performed in two species with very different resting heart rates, dogs and rabbits. Regional blood flow was studied by use of labelled microspheres. It is generally accepted that 15 μm microspheres which are trapped in arterioles of similar diameter give a good estimate of organ blood flow (Neutze, Wyler & Rudolph, 1968; Warltier, Gross & Hardman, 1976; Marcus, Heistad, Erhardt & Abboud, 1976). Small microspheres can travel through arterioles of appropriate diameters in some organs (Marcus *et al.*, 1976;

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Shu-Chien & Foun-Chung Fan, 1978). A drug causing vasodilatation could increase the number of microspheres delivered to a tissue by increasing its blood flow, and if 9 μm microspheres are used, increase the percentage of shunting if preferential dilatation occurs in the very small vessels. The sum of these two effects will determine the number of microspheres lodging in the tissue, and hence the apparent blood flow. Thus comparison of blood flow measurements made with 15 μm and 9 μm microspheres could reveal drug action on the very small (about 9 μm)-terminal arterioles. Two sizes of microspheres were thus used to find out whether the drug would act differently on smaller and larger arterioles.

Methods

Experiments were performed on nine pure bred Beagles of either sex weighing 14 to 16.5 kg, and on 34 New Zealand Red rabbits of either sex weighing 2.2 to 3.5 kg. All animals were anaesthetized with sodium pentobatrbitone (35 mg/kg) given intraperitoneally to dogs and intravenously to rabbits. After introduction of the tracheal cannula, the animals were allowed to breathe spontaneously. Arterial blood pressure was recorded via a cannula in the femoral artery and a Statham pressure transducer. The femoral vein was cannulated for introduction of heparin (2 mg/kg in dogs and 8 mg/kg in rabbits), for supplementary doses of anaesthetic when necessary (approximately 5 mg/kg every hour) and for administration of HWA 285 (3% solution in water).

In experiments in dogs, cardiac output was measured by the thermodilution method described by Fegler (1954), Klussman, Koenig & Lutcke (1959) and Goodyer, Eckhardt, Ostberg & Goodkind (1961) on the basis of the indicator-dilution principle. Cold saline (0°C, 0.4 ml/kg) was injected into the right atrium and blood temperature was recorded in the ascending aorta by means of a thermistor introduced via the brachial artery. A measuring unit (HZV 6560—Fischer-Hellige) and a Hellige polygraph was used for recording. Heart rate was counted from the ECG (standard lead II) and cardiac output and stroke volume (SV) were related to kg body weight. Total peripheral resistance (TPR) and cardiac work (CW) were calculated from cardiac output and mean arterial blood pressure (MBP). The left ventricular pressure curve was registered by means of a catheter-tip-manometer (PC 470, Millar, USA) introduced via the left carotid artery. The recorded left ventricular pressure signal was differentiated by an amplifier (Hellige) to obtain dP/dt values. An analog computer was used to determine a further contractility index

$$\frac{dP/dt}{(P-EDP)+C}$$

according to Kohler & Mescher (1973).

In experiments on rabbits, heart rate was taken from the blood pressure recording. Cardiac output and regional blood flow were estimated by use of carbon microspheres (15 ± 1.1 or 9 ± 0.9 μm, 3M Minnestoa Laboratories Ltd.) injected into the left ventricle via a catheter introduced through the left carotid artery. Microspheres were labelled with ^{46}Sc, ^{85}Sr or ^{141}Ce. Approximately 800,000 15 μm microspheres or 2,500,000 9 μm microspheres with a total activity of about 10 μCi (0.1 ml in 10% Dextran) were diluted to 1 ml with 6% Dextran and thoroughly mixed; the absence of clumps was confirmed periodically under the microscope. About 0.1 ml (the exact amount was determined by weighing the sample) of every injectate was placed in a test tube for radioactive counting, and exactly 0.9 ml was injected within 10 s into the left ventricle; 5 s before injection, blood was withdrawn from the femoral artery at a rate of 3.75 ml/min by means of a Braun infusion/withdrawal pump. The exact rate of withdrawal was calculated from the time of withdrawal (usually 45 s), weight of the blood withdrawn and its specific gravity which were ascertained for each sample. The total activity of withdrawn blood was measured for each isotope.

In each experiment, three values of blood flow were estimated: at rest, and during application of one or two different doses of HWA 285. In several experiments resting blood flow was estimated before and after drug application. Microspheres were injected 10 s after the injection of the drug, at the peak of the blood pressure response.

After the third isotope had been injected the animals were killed by an overdose of pentobarbitone, and samples of different organs taken into preweighed test tubes for measurement of radioactivity. The following samples were taken: three regions of brain cortex (frontal, parietal, occipital), the left ventricle of the heart, right and left kidney cortex, forelimb muscle and lungs. The samples were measured on a gamma ray spectrometer (Gamma Set 500, ICN Instruments, Belgium). The total activity in the lungs was measured as a % of the total amount injected. The measured activity in the individual tissue samples was corrected for crossover radiation, from Sc into Sr and Ce, and Sr into Ce, on the basis of the crossover radiation into inappropriate counting channels observed in samples of the injectates. The corrected activity was referred to 1 g of sample; cardiac output was calculated from the known total activity injected, and the withdrawn blood referred to ml/min withdrawal rate.

Blood flow was calculated from the activity of each sample and the blood activity derived from the withdrawn blood and expressed in ml 100 g^{-1} min^{-1}. Blood flow in any organ was calculated on the basis of the average of all samples measured. Peripheral resis-

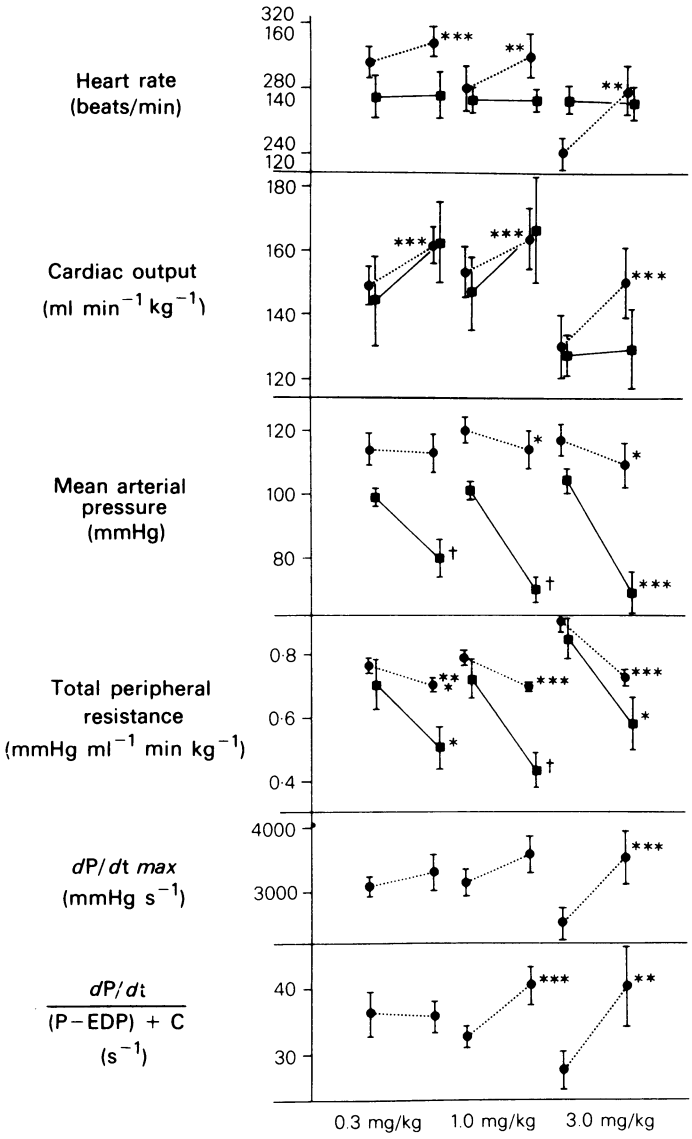

Figure 1 Heart rate, cardiac output, mean arterial pressure, total peripheral resistance and indices of cardiac contractility (dP/dt *max* and $dP/dt/(P-EDP) + C$ in dogs (●) and rabbits (■) in response to intravenous application of 0.3, 1.0 or 3.0 mg/kg HWA 285. The lines show a difference between control values and values during drug injection (solid lines = rabbits, interrupted lines = dogs). Each point represents mean values, vertical lines show s.e.mean. Dogs, $n = 7$ for 0.3 mg/kg, 8 for 1.0 and 3.0 mg/kg. Rabbits, $n = 5$ for 0.3 mg/kg, 6 for 1.0 and 3.0 mg/kg. Values significantly different from resting: $^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$; $^{\dagger}P < 0.001$.

tance and cardiac work were calculated from organ blood flow (or cardiac output) and mean blood pressure. Statistical evaluation was by means of unpaired, two-tailed t tests.

Results

Mean arterial blood pressure, heart rate, total peripheral resistance and heart performance in dogs

The results of the experiments in dogs are given in Figure 1. The changes in different parameters after application of each dose of drug were compared with resting values immediately before the application; consequently Figure 1 shows some variation in resting values. Resting heart rate varied between 120 and 148 beats/min, cardiac output between 130 and 153 ml min^{-1} kg^{-1} and mean arterial blood pressure between 114 and 120 mmHg. HWA 285 in the lowest dose (0.3 mg/kg) was without effect with the exception of a very slight increase in heart rate and cardiac output. In higher doses, 1.0 and 3.0 mg/kg, it produced a small but significant increase in heart rate, small but significant decrease in blood pressure and a marked increase in cardiac output. The latter was mainly due to the increase in heart rate since SV changed little. However, with the highest dose both dP/dt *max* and $dP/dt/(P-EDP) + C$ (contractility index according to Kohler & Mescher, 1973) were significantly increased. There was a significant decrease in peripheral resistance. The increase in CO and decrease in peripheral resistance resulted in no change in cardiac work. End-diastolic pressure varied between 1.3 and 3.3 mmHg at rest, and did not change significantly after the application of the drug (mean values being between 1.0 and −1.1 mmHg for the three doses).

Thus in the dog heart, HWA 285 had a dose-dependent positive chronotropic and inotropic effect, as well as dilator effect in the periphery which was shown in the dose-dependent decrease in total peripheral resistance.

Blood pressure, heart rate, total peripheral resistance and heart performance in rabbits

Unlike its effect in dogs, HWA 285 had neither a positive chronotropic nor an inotropic effect in rabbits: heart rate (246 to 282 beats/min at rest) and cardiac output (128 to 147 ml min^{-1} kg^{-1}) did not increase significantly even with the highest dose of HWA 285 (see Figure 1). There was a dose-dependent decrease in systolic, diastolic and mean blood pressure (the resting mean values for these variables were 124, 80 and 102 mmHg respectively), as well as a significant decrease in total peripheral resistance. Since the cardiac output did not change significantly, cardiac work decreased. This decrease was dose-dependent (from a resting value of 1.891 ± 0.187 $Jmin^{-1}$), the difference compared with resting values being 0.172 ± 0.028 with the lowest dose, 0.459 ± 0.225 with 1.0 mg/kg and 0.617 ± 0.190 $Jmin^{-1}$ with 3.0 mg/kg. The main effect of HWA 285 in rabbits was its vasodilator action in the periphery.

Regional blood flow and peripheral resistance in the brain, heart, kidney and muscle in rabbits

The results for regional blood flow and peripheral resistance in different organs are given in Figures 2, 3 and 4. The difference between flow in the right and left kidney was between 3 and 5% which indicates good mixing of microspheres (e.g. Johnston, 1975). Resting blood flow measured with 15 μm microspheres was within the range found in the rabbit by other authors (see Neutze *et al.*, 1968): 314 to 370 ml 100 g^{-1} min^{-1} in the heart, 45 to 56 ml 100 g^{-1} min^{-1} in the brain, 671 to 786 in the kidney and 3.9 to 5.6 ml 100 g^{-1} min^{-1} in skeletal muscle. With 9 μm micro-

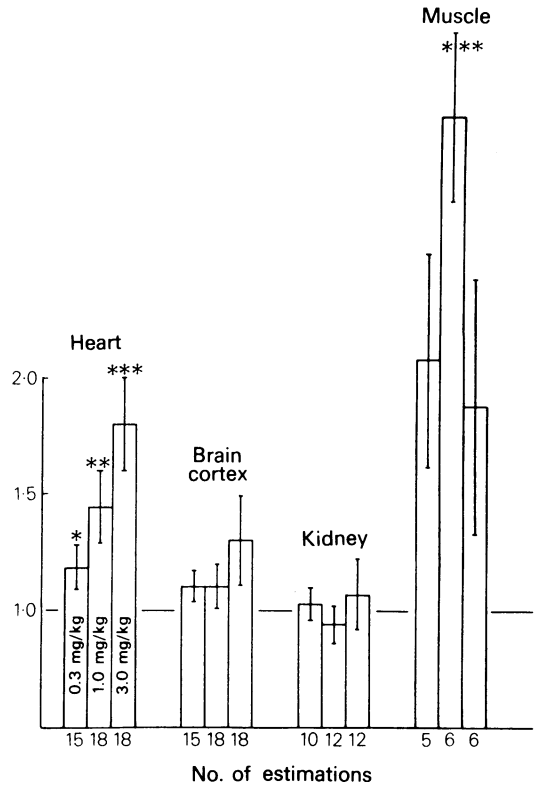

Figure 2 Changes in organ blood flow in rabbits measured with 15 μm microspheres. Resting flow = 1.0. Flow during administration of 0.3, 1.0 or 3.0 mg/kg HWA 285 expressed as a ratio of resting flow. Values significantly different from resting: $*P < 0.05$; $**P < 0.02$; $***P < 0.01$.

spheres, resting flow was occasionally lower, but on the whole the values were not very different (276 to 334 ml $100\,g^{-1}\,min^{-1}$ in the heart, 56 to 60 in the brain, 770 to 807 in the kidney and 3.4 to 5.3 in muscle). Some 9 μm microspheres had passed through the organs without being trapped, since the % of microspheres found in the lungs were higher for 9 μm (10.7 $\pm$ 0.8%) than for 15 μm (1.89 $\pm$ 0.23%) microspheres. When both sizes of microspheres were injected simultaneously, flow was 10% lower in the kidney and skeletal muscle with 9 than with 15 μm microspheres. When blood was sampled from the femoral vein, about 10% of 9 μm microspheres were found to have passed through the hind limb so that some shunting must have occurred. The proportion of untrapped microspheres was higher in the brain (20% on sampling from the jugular vein).

The effect of the drug was always compared with a resting blood flow which was taken as 1.0. The evaluation of the effect of the drug was based on the ratio between the flow after drug application and the control value (1.0) (Figure 2). With 15 μm microspheres, there was a dose-dependent and significant increase in blood flow in the heart, and a large increase in blood flow in skeletal muscle, although this was significant only with the middle dose because of the great variability. Blood flow in the brain increased with the lowest dose, the increase being greater with the highest dose, but this was not significant because of the variability of the effect. Blood flow in the kidney had a tendency to decrease with a higher dose. With 9 μm microspheres (Figure 3) blood flow in the heart showed a significant increase only with the lowest drug dose, unlike the results with 15 μm. There was no significant change in skeletal muscle blood flow with any dose, and there was a significant decrease in measured blood flow through the brain and the kidney. These results are probably due to the fact that dilatation of smaller arterioles allowed more of the microspheres to pass through the brain and, indeed, the percentage of 9 μm microspheres not trapped in the brain and found in the jugular vein was as high as 46% after administration of 3 mg HWA 285.

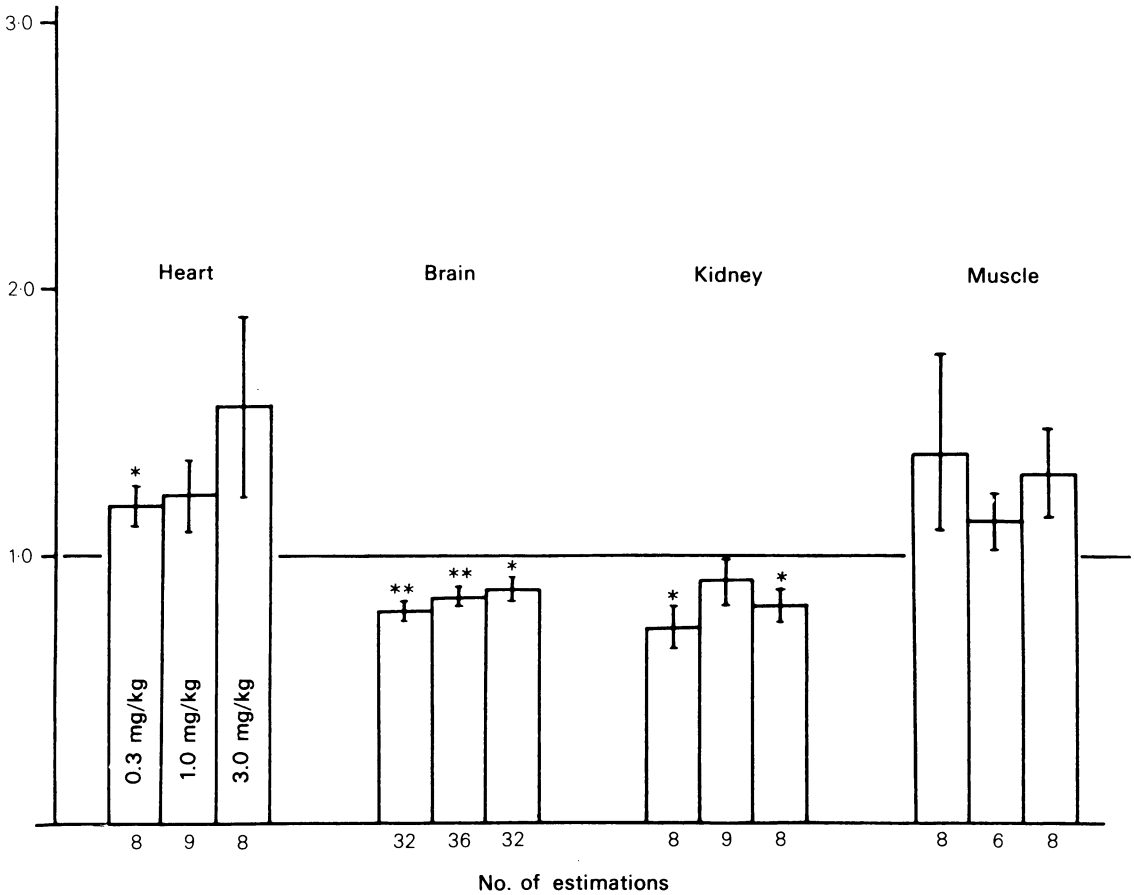

Figure 3 Changes in organ blood flow in rabbits measured with 9 μm microspheres. Details as in Figure 2.

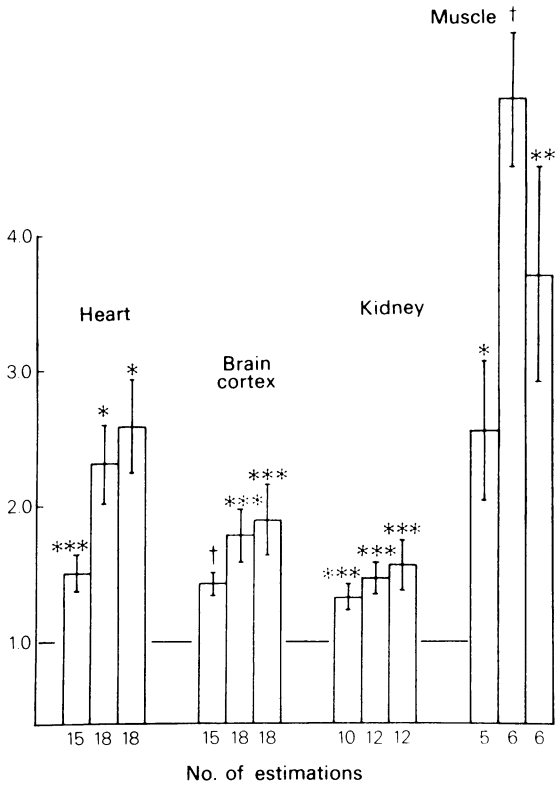

Figure 4 Ratio of peripheral resistance at rest to that during administration of different doses of HWA 285 in rabbits. Higher ratio indicates a decrease in the peripheral resistance. Blood flow was measured with 15 μm microspheres. Values significantly different from resting: $^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$; $^{\dagger}P < 0.001$.

Since all doses of HWA 285 produced a decrease in blood pressure it was necessary to evaluate their effect in individual organs in terms of peripheral resistance as well as blood flow. The results for 15 μm microspheres are shown in Figure 4. In this figure, peripheral resistance at rest was divided by resistance after drug application so that the increased ratio shows a proportional decrease in peripheral resistance. There was a dose-dependent decrease in peripheral resistance in the heart and skeletal muscle with 15 μm microspheres. However, the largest (3 mg/kg) dose produced a smaller increase in skeletal muscle than 1.0 mg/kg; this might be explained by the fact that dilatation was so great as to allow some microspheres to pass without being trapped. A highly significant and similar decrease in resistance in the brain was obtained with 1.0 and 3.0 mg/kg. The decrease in the resistance in kidney cortex was smaller, but significant. It is clear that in all organs studied, resistance was significantly decreased even with the lowest dose of the drug. Since there was shunting of the 9 μm microspheres, particularly after drug administration, the apparent values for peripheral resistance are not included; they showed a similar discrepancy with the 15 μm results as did flow, i.e. a smaller decrease in the peripheral resistance with drug administration for 9 μm microspheres than was measured with 15 μm microspheres.

Discussion

HWA 285 has been shown to have a positive chronotropic and inotropic effect in dogs as judged by the heart rate, and $dP/dt\ max$ and $dP/dt/(P-EDP) + C$. The absence of any change in stroke volume despite the positive inotropic effect could be due to a slight (non-significant) decrease in end-diastolic pressure which we observed with the highest dose of the drug. A dilator effect on the vessels in the periphery was seen in both dogs and rabbits. The decrease in peripheral resistance was greater in rabbits (30 to 40%) and was not dose-dependent while the decrease in dogs was much smaller (10 to 20%) and was dose-dependent. As a result of these actions there was only a very small hypotensive effect in dogs, but a marked dose-dependent hypotension (20 to 30%) was observed in rabbits. How can these differences be explained?

As far as the differences between the chronotropic and inotropic effects are concerned, it is possible that the drug has a direct effect on the myocardium in the dog which is absent in the rabbit. A more likely explanation lies in the differing degree of reserve performance available in the hearts of dogs and rabbits. The dog is capable of large increases in heart rate and stroke volume, producing considerable sustained increases in cardiac output, which is necessary for the powers of endurance of the animal. The rabbit, on the other hand, has a very high resting heart rate, and the mean control heart rate of 273 beats/min seen in our experiments is quite close to the maximum possible for this species. Because the rabbit has the smallest heart weight in relation to body weight of any mammal (Latimer & Sawin, 1956; Poupa, Rakusan & Ostadal, 1970) the maximum stroke volume is obviously relatively small. The cardiac output reserve is therefore considerably smaller than that of the dog and the animals are probably using a greater proportion of their maximum heart rate and cardiac output capacity already at rest.

The drug produced a greater decrease in the total peripheral resistance in the rabbit than in the dog which was probably due to a greater vasodilatation in the rabbit and consequently greater decrease in blood pressure which could not be compensated for by the baroreceptors since baroreceptor reflexes in this species stay activated at much lower pressures than in dogs (Koch, 1931).

In contrast, dogs were able to compensate partially for the blood pressure drop which would result from reduced peripheral resistance by increasing heart rate and cardiac output. Stroke volume did not change, in spite of significantly increased myocardial contractility, probably due to a reduction in the end-diastolic pressure and thus end-diastolic volume. We did not measure contractility of the heart in the rabbit, but the increase in cardiac output in the absence of the increase in the heart rate with lower doses of HWA 285 indicates that some positive inotropic effect must have been present.

The most remarkable effect of HWA 285 was the increase in regional blood flow observed with 15 μm (range 13 to 18 μm) microspheres, particularly in the heart and in skeletal muscle. This contrasted with much smaller changes in apparent blood flow when 9 μm (range 7 to 10 μm) microspheres was used. It is generally accepted that the number of microspheres trapped in the vessels of the appropriate diameters is related to flow, provided trapping is complete and this has recently been confirmed by direct observations by Wiedeman (1979). The percentage of trapped microspheres in most organs was 99% in the case of 15 μm microspheres (Shu-Chien & Foun-Chung Fam, 1978). These microspheres also give very reproducible data for blood flow measurement in the brain (Marcus *et al.*, 1976) and in the heart (Warltier *et al.*, 1976). The blood flow values found in our experiments are in good agreement with the values obtained in the rabbit by Neutze *et al.* (1968) using 50 μm microspheres, which are fully trapped everywhere. So on the whole, 15 μm microspheres give reliable information about blood flow through arterioles in this diameter range. Smaller microspheres would obviously be trapped in smaller vessels. However, Wiedeman (1979) observed that some microspheres can be forced through smaller vessels by the contractile activity of vascular smooth muscles, and several authors have reported a certain degree of shunting with 9 μm microspheres (in the brain 8%, Marcus *et al.*, 1976; 17%, Shu-Chien & Foun-Chung Fan, 1978). If shunting occurs at rest, measured resting blood flow should be lower with microspheres that are not trapped (9 μm) than with microspheres that are (15 μm). This was true only for skeletal muscle in our experiments. Resting flow in the heart was similar with 15 and 9 μm microspheres, and was higher with 9 μm microspheres in the brain. This is in a sharp contrast to our finding of 20% microspheres in the jugular vein. This high percentage could be partly attributed to shunting in the extracerebral circulation, as suggested by Marcus *et al.* (1976) for dogs.

The finding of a higher measured cortical blood flow with 9 μm microspheres might be explained by the lower tendency of these microspheres to be affected by axial streaming (Phibbs & Dong, 1970). If the cortex is supplied more by vessels which branch off sharply from their parent vessels, than in any other region of the brain, 9 μm spheres may be better able to reach the cortex than 15 μm spheres, since they are more evenly distributed across blood vessels and therefore more likely to execute sharp turns into side branches.

Increased blood flow in the heart, brain and skeletal muscle measured by 15 μm microspheres during administration of 3 mg/kg HWA 285 were very similar to maximal functional hyperaemia occurring in these tissues (see Folkow & Neil, 1971).

The increase in flow measured by 9 μm microspheres was much smaller. In the brain, measured blood flow with 9 μm microspheres was reduced by the drug, rather than increased, as it was with 15 μm. In cardiac and skeletal muscle, the HWA 285-induced increases in apparent blood flow measured with 9 μm were much smaller than with 15 μm microspheres. In the kidney, measured blood flow with 9 μm was reduced rather than relatively stable, as with 15 μm. This is consistent with the increased % of 9 μm microspheres in outflowing blood after administration of the drug. These differences are probably due to increased passage of 9 μm microspheres through all these tissues during the effects of HWA 285. This indicates a profound effect of the drug on the diameter of certain vessels, normally approximately 7 to 10 μm in diameter (as the s.d. of the microsphere diameter is $\sim$ 1 μm, 7 to 10 μm vessels should exclude a high or considerable proportion of the microspheres) which can dilate to at least 9 μm (allowing about half of the microspheres to pass). These vessels must also not join any narrow vessels distally; or trapping would occur later.

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