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ACTIONS OF SUBSTANCE P ON THE GENERAL, PULMONARY, AND CORONARY HAEMODYNAMICS AND METABOLISM OF INTACT DOGS

BY

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Substance P is a polypeptide with vaso-active properties, first isolated by von Euler & Gaddum (1931), which is found in nervous tissue and gut. The basic pharmacology of substance P and its differentiation from other vaso-active substances has already been described (Boissonas, Franz & Stürmer, 1963). The present study was carried out in order to find the effects of substance P on the general and coronary haemodynamics of intact anaesthetized dogs.

METHODS

Apparently healthy dogs, unselected by age, breed, or sex, were premedicated with morphine sulphate (3 mg/kg) subcutaneously; 1 hr later they were anaesthetized by the intravenous injection of "dial-urethane-pentobarbitone" 0.25 ml./kg. ("Dial-urethane-pentobarbitone" contains allobarbitone 100 mg/ml. and monoethylurea and urethane, each 400 mg/ml. Pentobarbitone 60 mg/ml. was used as a 1:1 diluent.)

General and coronary haemodynamics were studied before and after the intravenous injection of substance P (Hoffmann-LaRoche); the amount given, in 5–7 ml. of water, averaged 0.15 mg/kg, and was sufficient to maintain a reasonably steady state of hypotension and tachycardia during the remainder of the experiment. The substance P was stated to contain 300 von Euler units/mg.

Each study began 1 hr after the intravenous anaesthetic; a cuffed endotracheal tube was placed, and needles passed through the skin into the femoral arteries. These were connected to a manifold system which allowed frequent pressure-measurement or blood sampling.

In the ten group A dogs, a small incision was made to isolate the superficial neck veins; through them cardiac catheters were placed by fluoroscopy in a main branch of the pulmonary artery, and in the coronary sinus. The position of the latter catheter was confirmed by the characteristic X-ray silhouette, and by the aspiration from it of blood which was substantially lower in oxygen content than that from the pulmonary artery. In these animals, cardiac output was frequently measured by dye-curve before and after the injection of substance P. The tracer used was indo-cyanine green, the transducer was a Gilford densitometer. The computation of cardiac output was by the "forward triangles" method (Hetzel, Swan, DeArellan & Wood, 1958). In these dogs too, mean coronary flow over 10 min was measured by the nitrous oxide saturation method using the Fick principle.

In the ten dogs of group B, cardiac output was measured by the direct Fick method, with a 5 min expired air collection, at the mid-point of which simultaneous blood samples were drawn from the pulmonary and femoral arteries.

In the five group C dogs, coronary sinus blood flow was measured by thermodilution flowmeter (Afonso, 1966); in this instrument, heat produced by an electrically heated coil is distributed uniformly

in the blood stream by a stirrer, and the temperature change is measured downstream by a thermistor; the device is mounted at the tip of a No. 8 cardiac catheter, and measures flow at the cross-sectional area where the thermistor is placed. This instrument was put into a neck vein and placed in the coronary sinus by fluoroscopy; its position was confirmed by this characteristic X-ray silhouette, and its performance assessed after the primary experiment by the injection of a coronary vasodilator (bradykinin), and constrictor (pituitrin). Finally, the siting of the instrument was inspected at autopsy.

Blood oxygen, carbon dioxide, and nitrous oxide contents were measured in a manometric Van Slyke machine. The Scholander apparatus was used to analyse expired air samples for oxygen and carbon dioxide; intravascular pressures were measured by Statham strain-gauge, electrically integrated for mean values, and recorded on a "direct-writing" instrument.

Ventricular work and vascular resistances were derived by accepted formulae (Wiggers, 1954). Arterial glucose was measured enzymically (Huggett & Nixon, 1957), and non-esterified fatty acid by micro-titration (Trout, Estes & Friedberg, 1960). The control measurements were followed by the intravenous injection of substance P. In a few other animals the effect of atropine or angiotonin on the substance P results was examined. In these dogs, only heart-rate and vascular pressures were noted. It will be seen that in general each dog acted as its own control.

The general methods used, and their reproducibility, have been previously reported (Maxwell, Castillo, White, Crumpton & Rowe, 1959; Maxwell, 1966). Statistical comparison of the group means was done by Student's *t* test; statistical significance was accepted at the 5% level.

The general pattern of the study then was to measure cardiac output by dye-curve before, and during the 20 min after the injection; the direct Fick cardiac output was obtained before and during the 5 min after injection; mean coronary flow over 10 min (N_2O Fick) was obtained before, and 7–10 min after the injection; coronary sinus flow (thermodilution flowmeter) was measured before and continuously for 20 min after the injection.

RESULTS

The acute effects of substance P on heart rate and systemic and pulmonary arterial pressures are shown in Fig. 1; depression of systemic pressure is greatest in the first 2 min, and continues, with tachycardia, for between 20 and 25 min. A typical response

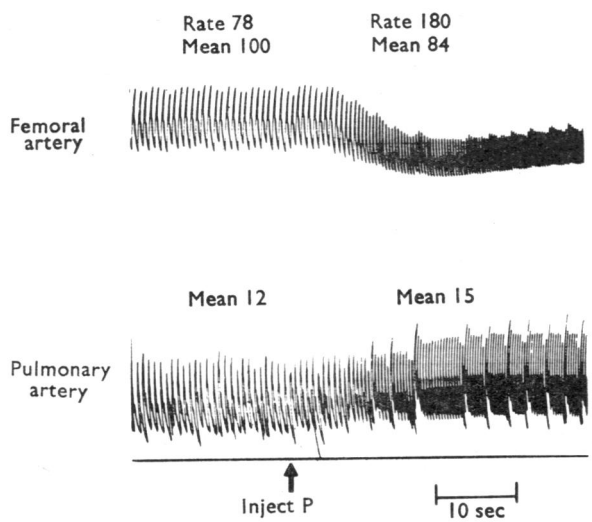

Fig. 1. Effect of substance P on heart rate and vascular pressures.

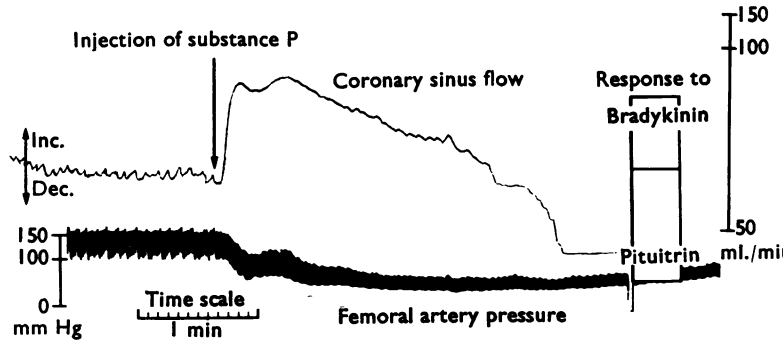

Fig. 2. Effect of substance P on coronary sinus flow. The later response in the same animal to bradykinin (15 μg) and pituitrin (2 pressor units) is also shown.

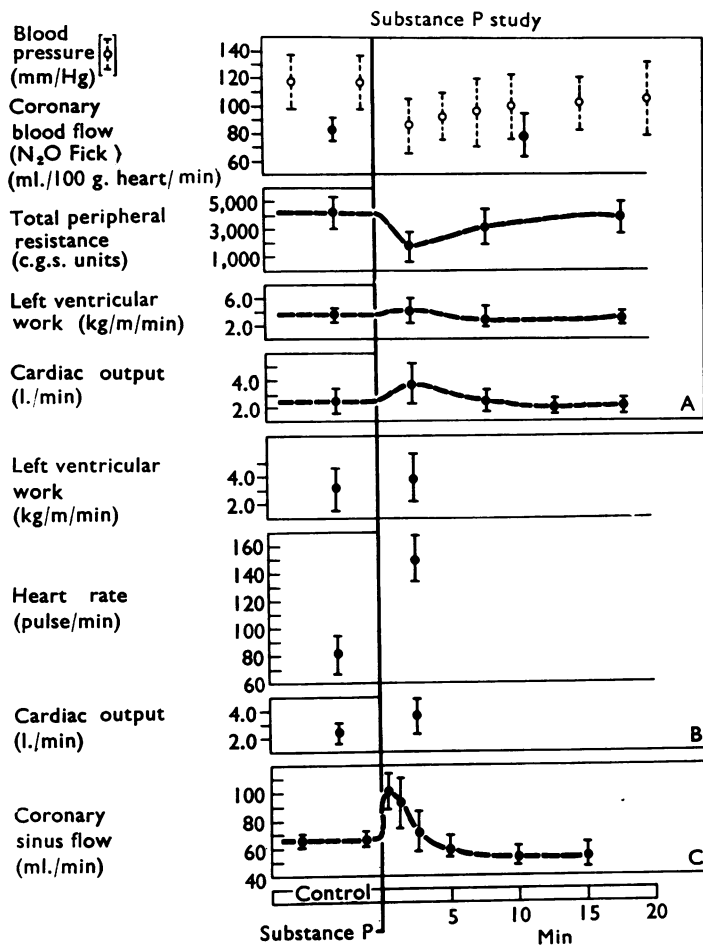

Fig. 3. Summary of results from group A, B and C dogs; values are group means with S.D.

of coronary sinus flow (by thermodilution flowmeter) is shown in Fig. 2 (group C dogs). The combined results of the major parameters are shown in Fig. 3, which correlates, on a time basis, the results from all three groups of animals.

The results of sequential measurements of cardiac output by dye-curve (group A animals) reveals a pattern of transient, but statistically significant, increase, followed by a decline to less than control value. The phase of increased cardiac output was further explored in the group B animals, using the Fick principle, and Tables 2 and 3 add further information concerning this aspect; from them it is seen that the stage of increased cardiac output is associated with an increase in whole-body oxygen consumption and carbon dioxide production, the change in the latter being more marked, thus giving rise to the statistically significant increase in exchange-ratio (R.Q.); it is dubious, however, whether this R.Q. change is physiologically significant. The increase in pulmonary arterial oxygen content

TABLE 1

GENERAL METABOLISM (GROUP B ANIMALS)

Figures are group means with S.D. Gas volumes are S.P.T.D. * *P* value of difference 0·05 or less.

Factor	Control	Experimental
Respiratory exchange (l./min)	2·5 ± 0·6	3·1 ± 0·7*
Oxygen consumption (ml./min)	77 ±14	84 ±16*
Carbon dioxide production (ml./min)	58 ±12	69 ±13*
Exchange ratio (R.Q.)	0·75± 0·08	0·81± 0·07*
Arterial oxygen content (ml./100 ml.)	16·3 ± 1·3	17·0 ± 1·9
Mixed venous oxygen content (ml./100 ml.)	12·8 ± 2·0	14·6 ± 1·8*
Δ Arterial—mixed venous oxygen (ml./100 ml.)	3·5 ± 1·0	2·4 ± 0·9*
Mixed venous carbon dioxide content (ml./100 ml.)	51·5 ± 4·5	49·6 ± 3·1*
Arterial carbon dioxide content (ml./100 ml.)	48·1 ± 4·2	46·4 ± 4·0*
Δ Mixed venous—arterial carbon dioxide (ml./100 ml.)	3·4 ± 0·8	3·2 ± 0·8

TABLE 2

GENERAL HAEMODYNAMICS (GROUP B ANIMALS)

Figures are group means with S.D. * *P* value of difference 0·05 or less.

Factor	Control	Experimental
Cardiac output (l./min)	2·3±0·8	3·5±1·4*
Heart rate (beats/min)	80±14	150±17*
Stroke volume	29±10	23±15
Femoral pressure (mean mm Hg)	100±21	83±17*
Pulmonary arterial pressure (mean mm Hg)	12±4	14±6*
Left ventricular work (kg/m/min)	3·1±1·6	3·9±1·8*
Right ventricular work (kg/m/min)	0·39±0·2	0·80±0·2*
Total peripheral resistance (c.g.s. units)	5,791±2,130	3,158±1,121*
Total pulmonary resistance (c.g.s. units)	473±67	347±150*

is similar to that for forearm blood in man (Lofström, Pernow & Wahren, 1965), and the resulting decrease in whole-body oxygen extraction (Δ arterial–mixed venous oxygen) is a principal mathematical association of the calculated increase in cardiac output.

The coincidence of result by two separate methods would tend to exclude serious errors in each, and makes it likely that the finding of increased cardiac output is a true one.

The increase in pulmonary arterial pressure (Table 2) is quantitatively small, although statistically significant; the change was not related to one in pulmonary "capillary" pressure, which, as in man given substance P (Pernow, 1963), was unchanged (before, group mean, 2.5 ± 1.5 mm Hg, after, 2.6 ± 2.0 mm Hg). There was no statistically significant change in glucose level which before substance P was 88 ± 18 mg%, and after, 84 ± 20 mg%; similarly, non-esterified fatty acid changes were unaffected (before 0.43 ± 0.15 m-moles/l., after 0.42 ± 0.19 m-moles/l.).

The response of the coronary sinus flowmeter (Figs. 2 and 3) suggests that the coronary vasodilator effect of substance P is rather transient. The calibration of the flowmeter is logarithmic (Fig. 2), so that the value for decrease in flow is approximate; however, the figures for mean coronary flow over 10 min by the independent nitrous oxide Fick principle (Table 3) tend to confirm such a decrease. The time-relationship of the latter

TABLE 3

CORONARY HAEMODYNAMICS, MYOCARDIAL OXYGEN AND CARBON DIOXIDE METABOLISM

The "index of efficiency" is the ratio of left ventricular work to the cardiac oxygen consumption. Figures are group means with S.D. * P value of difference 0·05 or less.

Factor	Control	Experimental
Coronary flow (ml./100 g heart/min)	84 ±9	78 ±17
Coronary vascular resistance (arbitrary units)	1·44±0·28	1·20± 0·24
Coronary sinus oxygen (ml./100 ml.)	5·8 ±1·5	5·2 ± 2·0
Δ Arterial—coronary sinus oxygen (ml./100 ml.)	11·2 ±1·4	11·7± 1·8
Coronary sinus carbon dioxide (ml./100 ml.)	52·4 ±4·8	49·5 ± 6·1*
Δ Coronary sinus—arterial carbon dioxide (ml./100 ml.)	8·64±2·4	8·5 ± 1·5
Cardiac respiratory quotient	0·76±0·14	0·73± 0·07
Cardiac oxygen consumption (ml./100 g heart/min)	9·6 ±1·8	9·3± 2·7
Cardiac carbon dioxide output (ml./100 g heart/min)	7·8 ±1·8	7·2 ± 1·9
"Index of efficiency"	0·41±0·11	0·32± 0·05*

measurement to the general study should be carefully noted, because the derived results for myocardial metabolism, etc., in Table 3 occur at a stage of decreased coronary flow by the flowmeter technique, and in the stage of lowered cardiac output, as shown by the dye-curve method.

In this, as in other studies, the most constant findings are of tachycardia and hypotension, both of which persisted for between 20 and 25 min after injection; a direct action of substance P on the vessels has been demonstrated in the rabbit-ear preparation (Holton & Holton, 1952), and in avian vessels (Buñag & Walaczek, 1963); in the chicken

at least, the hypotensive effect is still present after decapitation, which suggests that the medullary centres are not essential for the hypotensive response, which also persists after vagotomy or atropine ; it has also been shown (Buñag & Walaczek, 1963) that substance P does not release histamine as a secondary hypotensive agent. The most likely cause of the hypotension then is a direct dilating effect on the blood vessels ; the present study does not disprove this conclusion, but it does suggest that the action may apply also to the pulmonary and coronary vascular beds.

The tachycardia is another constant response, and, as Figs. 1 and 2 show, this is coincident with the fall in blood pressure, which perhaps suggests a baroceptor response. The inverse relationship between heart rate and blood pressure (Table 2), follows Marey's law (Marey, 1859) and is further evidence of the baroceptor association of the finding. In two other dogs studied, the pressure and rate effects of substance P were readily reversed by injecting enough angiotonin to restore blood pressure—again evidence of a baroceptor mechanism. In two other dogs the effects of large doses of atropine on the effect of substance P was examined. Atropine did not inhibit the tachycardia (for example, control rate 66/min, after atropine 115/min, after substance P 150/min) or the degree of hypotension. This result does not exclude a baroceptor origin of the tachycardia, although some degree of sympathetic activation might contribute to the result (compare Glick & Braunwald, 1965). A direct cardiac effect of substance P in causing tachycardia has apparently been excluded, at least for the isolated auricle or papillary muscle, by Vogler *et al.* (1963). In general, then, the results now reported confirm the suggestion of a direct effect of substance P on the vessels with a resultant pressure-related tachycardia, presumably baroceptor induced.

The pattern of cardiac output response now described probably explains the varying reports concerning this measurement. Thus Düner & Pernow (1960) showed only a minor (and apparently statistically insignificant) increase in cardiac output ; a later report (Pernow, 1963), however, demonstrated a definite increase. As Fig. 3 shows, the result found will depend on the time-relationship of the measurement to the injection of substance P ; the argument of Düner & Pernow (1960), that increased cardiac output with substance P is " due to the tachycardia " can be supported from the data given for the group B dogs ; this correlation, however, holds only for the first 6 to 7 min after the substance P injection. The later decline in cardiac output (Fig. 3) occurred while the heart was still fast, and the blood pressure lowered. Accordingly, as a general statement, the changes in cardiac output are not consistently associated with the increased heart rate. This is perhaps not unreasonable, because, in similary prepared dogs, induced tachycardia did not affect cardiac output (compare Maxwell, Castillo, White, Crumpton & Rowe, 1958). As Fig. 3 suggests, there is also a correlation between the depression of systemic pressure and the cardiac output response, although again, this does not hold very well after the first 5 min.

The figures derived from the primary observations of cardiac output and vascular pressures again vary with the time after the injection. Clearly enough (Fig. 3) the early increase in cardiac output is more than enough to offset the initial steep decline in systemic pressure, so that left ventricular works shows a transient net increase. Both the primary factors (pressure and flow) are concerned in keeping left ventricular work below control values thereafter. Again, in terms of calculated peripheral resistance,

substance P exerts its principal effect in the first few minutes after the injection, although the value for peripheral resistance continues to be reduced during the total study time. The pattern of pulmonary vasodilatation, in terms of calculated pulmonary vascular resistance, will show a similar pattern. Stroke volume is substantially unchanged when cardiac output is at its peak (Table 2), but because tachycardia continued through the study period, will reach a nadir when cardiac output is low. The presence of tachycardia and systemic hypotension would seem to be evidence of continuing substance P activity during the study period. If this is accepted, then the effects on general haemodynamics are biphasic, in the sense that factors such as cardiac output and left ventricular work will vary according to the time after injection at which the measurement is made. The maintenance of glucose and fatty acid levels at control values is probably of little consequence except to suggest that there is no appreciable release of catecholamines (Klein, Estes & Bogdonoff, 1961); the unchanged haemoglobin levels could be similarly interpreted.

The values derived by the thermodilution flowmeter (Figs. 2 and 3), show that substance P increases cardiac venous flow for several minutes; this effect is followed by a return of the flow to control or lesser level; Fig. 2 also shows an example of the response to bradykinin in the same dog. The bradykinin was given to check the response of the flowmeter, and to provide some sort of pharmacological comparison. Inspection of Fig. 2 shows that the increase in coronary flow is largely independent of the degree of hypotension or tachycardia; in this it is an exemplar of all the dogs in group C; Fig. 3 suggests a relationship with cardiac output, or left ventricular work; however, because the data were derived from separate groups of animals, this association should perhaps be interpreted with caution; it is fair, however, to state that such an association of coronary flow and cardiac output has been observed by others (Foltz, Page, Sheldon, Wong, Tuddenham & Weiss, 1950) in similarly prepared animals.

The increase in coronary flow is so transient that there is obvious difficulty in finding out what happens to myocardial oxygen and carbon dioxide exchange at this stage. On the assumption that the time and pattern of response were constant, small samples of blood were drawn from the coronary sinus and femoral arteries of two dogs at the presumed time of coronary vasodilatation. These samples were examined for oxygen saturation by haemo-reflexion. The results for the coronary sinus samples showed that the control saturation (20%), increased to a maximum of 40% 30 sec after the injection, and returned to near control level (25%) by 1 min; accordingly, the period of decreased myocardial oxygen extraction (Δ arterial–coronary sinus oxygen) would be very short.

The results concerning coronary flow by the Fick principle (group A dogs, Table 3) clearly would be those occurring in the phase of a return of coronary sinus flow to control, or lower values in the group C dogs (Fig. 3). The small decrease in coronary flow shown for the group A dogs would tend to confirm this statement. Accordingly, statements concerning myocardial oxygen and carbon dioxide metabolism should be interpreted against this background. In any case, the changes found again occurred when mean systemic pressure was reduced; thus calculated coronary vascular resistance in the group was significantly reduced. It is obvious from Figs. 2 and 3 that such a reduction is a reasonably constant phenomenon with substance P and will be most marked in the first few minutes after injection.

Cardiac oxygen consumption is the product of coronary flow and myocardial oxygen extraction (Δ arterial–coronary sinus oxygen); the limited data from the flowmeter (for example, Fig. 2), and haemoreflexion oxygen studies already referred to, suggest that in the first few minutes after injection cardiac oxygen consumption would be increased, principally because of the increase in coronary flow; by the stage at which coronary flow is measured in the group A dogs—from 6 to 7 min after injection—there are only minor changes in coronary flow and myocardial oxygen extraction, so that the cardiac metabolic rate for oxygen (cardiac oxygen consumption) was unchanged; by this stage, as shown in Fig. 3, left ventricular work is lower than control so that the "index of efficiency"—the oxygen cost of left ventricular work—is significantly reduced; although indirectly derived, this "index" bears a reasonable relationship to cardiac efficiency as calculated by Joule's law (compare Maxwell, Elliott & Kneebone, 1962); in the phase of increased coronary flow by flowmeter, which more or less coincides with the phase of increased left ventricular work (Fig. 3), this reduction in cardiac efficiency is probably less marked.

As in the study of general haemodynamics, substance P then has a vasodilating effect on the coronary circulation; this effect is transient, although, as assessed by continuing hypotension and tachycardia, the polypeptide is pharmacologically active throughout the period of study. There seems to be a correlation between the increase in coronary flow and the phase of increased cardiac output and left ventricular work (see Fig. 3), although these correlations are associative and not necessarily causal, because the comparisons are derived from two separate groups of animals.

In general then, substance P dilates the systemic, pulmonary, and coronary vascular beds. The effect is transient, and biphasic in the sense that cardiac output and coronary sinus flow return to below control values while hypotension is still present. The general profile of response is similar to that of other vasodilator polypeptides such as bradykinin (compare Maxwell *et al.*, 1962), and is probably the result of a primary effect on the blood vessels. The transience of the response is such that studies of other circulatory subdivisions—the cerebral and splanchnic circulations—should employ instruments capable of a rapid response.

SUMMARY

1. Twenty-five intact anaesthetized dogs were studied before and after an intravenous injection of substance P 0.15 mg/kg; in ten dogs cardiac output (dye-curve) was measured before and for 20 min after the injection; coronary flow (N_2O Fick) was also measured; in ten dogs cardiac output (Fick) and derivatives were measured in the 5 min after injection; in five dogs coronary sinus flow was measured by thermodilution flowmeter before and for 20 min after injection of substance P.

2. Substance P caused tachycardia and hypotension for 20 min after its injection; in the first 5 min cardiac output and left ventricular work were increased. This coincided with increased coronary sinus flow; the latter, and the cardiac output, then reverted to less than control values. There was a constant reduction in coronary vascular resistance and cardiac efficiency.

3. Substance P dilates the coronary as well as the pulmonary and systemic circuits; it does not seem to release catecholamines.

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