J. Physiol. (1980), **302**, *pp.* 31–47
With 6 text-figures
Printed in Great Britain

EFFECTS OF SUBSTANCE P ON CAROTID CHEMORECEPTOR ACTIVITY IN THE CAT

BY D. S. McQUEEN

From the Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

(*Received 8 May 1979*)

SUMMARY

1. The influence of substance P (SP) on spontaneous chemosensory discharge and on responses of the carotid chemoreceptors to various drugs has been investigated in pentobarbitone anaesthetized cats in which chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve.

2. After an initial slight inhibition during the first 5–15 sec following the injection, SP (0·1–100 μg I.A.) caused a dose-related increase in discharge which lasted for 45–300 sec in artificially ventilated cats, discharge being increased by about 50% on average. The increase was of shorter duration when the animals were allowed to breathe spontaneously.

3. The delayed increase in discharge was not secondary to the hypotension caused by SP, nor was it entirely due to changes in bronchomotor tone resulting from direct or indirect actions of SP, although such changes contributed to the response. It was not possible to determine whether the excitation was due to a direct effect of SP on the chemoreceptors.

4. Chemosensory excitation evoked by NaCN (5 μg I.A.) was potentiated during I.A. infusions of SP and also 10–20 min after SP (10 μg I.A.) had been injected. In contrast, responses to ACh (50 μg I.A.) were inhibited. These effects may be due to a nicotinic-blocking action of SP on the carotid chemoreceptors. It was also found that the inhibitory action of dopamine (5 μg I.A.) was reduced during SP infusion whereas that of 5-HT (10 μg I.A.) was potentiated.

5. A sample of crude SP had effects on spontaneous chemoreceptor discharge and responses to NaCN and ACh which were qualitatively similar to those obtained using synthetic SP.

6. The physiological significance of the results is discussed and it is concluded that the interpretation depends upon whether or not SP is present in the cat's carotid body.

INTRODUCTION

The carotid body type 1 cell is considered by Pearse (1969) to be a member of the APUD cell series (i.e. cells which share the characteristics of *a*mine and amine *p*recursor *u*ptake and *d*ecarboxylation, and polypeptide secretion). Pearse suggested that the type 1 cell secretes a low molecular weight polypeptide, which he tentatively named 'glomin'. Histological evidence has since been obtained which indicates

0022-3751/80/7970-0194 $07.50

that polypeptide or protein-containing granules are present in mammalian carotid body cells (Capella & Solcia, 1971; Pearse, Polak, Rost, Fontaine, Le Lièvre & Le Douarin, 1973).

Immunohistochemical evidence (Hökfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygåards & Pernow, 1977; Cuello, 1978) shows that SP-like material is present in the peripheral endings of various sensory nerves and it has also been reported that synthetic SP can stimulate sensory nerve endings (Juan & Lembeck, 1974).

As there is a possibility that carotid body cells secrete a polypeptide, and it is known that SP-like material is associated with some peripheral sensory nerve endings, it seemed worth determining what effect SP has on the cat carotid chemoreceptors. A preliminary report on some of the results has been made to the Physiological Society (McQueen, 1978*a*).

METHODS

Most of the details have been described previously (McQueen, 1977; Docherty & McQueen, 1978) and only a brief summary follows. Experiments were performed on cats of either sex weighing between 2·2 and 3·7 kg (median weight 2·8 kg, $n = 11$) which were anaesthetized with pentobarbitone sodium (42 mg/kg I.P.) supplemented approximately every 1·5–2 hr during the experiment by I.V. administration of 10 % of the initial dose. Blood pressure was recorded from one femoral artery and the other femoral artery was cannulated for arterial blood sampling. Rectal temperature was maintained at 38 ± 0·5 °C and the bladder drained at regular intervals.

A sinus nerve was dissected free from surrounding tissues, cut centrally, and the electrical activity of single or multiple chemoreceptor units (two to four active units preferred) recorded from the peripheral nerve using bipolar platinum electrodes and an a.c. amplifier (Neurolog; Digitimer). The ganglio-glomerular nerves were cut in order to prevent changes in sympathetic nerve activity influencing chemoreceptor discharge (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961*a*). For part of some experiments the animals breathed spontaneously and respiration was recorded via an integrating pneumotachograph (CS3c; Mercury Electronics) which was used in conjunction with a time clock (2112; Palmer) to provide a cumulative record of total volume inspired over a period of 30 sec. A 'staircase' tracing was obtained on the pen recorder (MX6; Lectromed) giving a breath-by-breath record of respiration with the over-all height being proportional to the respiratory half-minute volume. For the rest of the experiments the animals were artificially ventilated with room air and usually paralysed by gallamine triethiodide (3 mg/kg I.V.). End-tidal CO_2 was continuously monitored by an infrared CO_2 analyser (Med 1A; Grubb Parsons) and the P_{a,CO_2}, P_{a,O_2} and pH of femoral arterial blood samples measured using a gas monitor (BMS3 with PHM71 meter, Radiometer).

Nerve activity was recorded on magnetic tape (Tandberg 100; frequency response d.c. to 1250 Hz) and subsequently analysed with the aid of a computer (PDP-8; Digital Equipment Corporation) in order to provide data concerning discharge frequency. The average ($\bar{x}$ ct/sec) and total count (Σx) were calculated for each response after its duration (t sec) had been determined. Responses were expressed as a change from control level by subtracting the appropriate control or background discharge (i.e. as $\Delta\Sigma x$). Data from different experiments were pooled and the results presented as the mean ± S.E. of mean.

Drug solutions (0·1 ml.) were injected into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded and washed in with 0·2 ml. modified Locke solution which had been bubbled with 5 % CO_2 in air in a water bath at 37 °C; the wash solution had no effect on spontaneous chemoreceptor discharge. The catheter was introduced into the common carotid artery via the lingual artery, and its tip lay about 2 cm caudal to the carotid bifurcation. Injections were made over a 2 sec period and at least 20 min allowed to elapse between doses of SP. Drug *infusions* were made through a second catheter situated in the common carotid artery at its junction with the superior thyroid artery (through which the catheter was introduced) using an infusion pump (Braun) which delivered 0·5 ml./min of drug

solution. Injections of stimulants NaCN or acetylcholine (ACh)) into either of the two carotid catheters evoked very similar chemoreceptor responses.

Drugs were prepared in modified Locke solution (NaCl 6·0 g; KCl 0·42; $CaCl_2$ 0·24; Tris base 6·0 g; *N*-HCl, 39 ml.; distilled water to 1 l.; pH 7·41 at 37 °C). Doses referred to are those of the salts. Glassware used for SP was silanized. The drugs used in this study were: pentobarbitone sodium, gallamine triethiodide (May & Baker); acetylcholine iodide, atropine sulphate, sodium cyanide (B.D.H.); dopamine hydrochloride (Koch-Light); 5-hydroxytryptamine creatinine sulphate, synthetic Substance P (Sigma; Beckman).

A sample of SP which had belonged to Professor Gaddum was kindly given to me by Dr T. B. B. Crawford of this department. The 1 mg sample of crude SP had been extracted from horse intestine, adsorbed onto lactose and stored in a nitrogen-filled sealed ampoule. Its activity was 75 units/mg, which is approximately equivalent to 0·38 μg synthetic SP/mg extract (Euler, 1977).

RESULTS

Injection of SP

In artificially ventilated cats SP injected close-arterial to the carotid body in a dose of 0·1–100 μg usually caused a slight inhibition of spontaneous chemoreceptor discharge during the first 5–15 sec after the injection, an effect which did not appear to be dose-related. The initial inhibition was followed by an increase in discharge which, although not substantial, lasted for 45–300 sec and was dose-related (see Figs. 1 and 2). Low doses of SP (0·1–2·5 μg) did not always cause a delayed increase in discharge: there was often no change. Tachyphylaxis seemed to develop to the delayed increase in chemoreceptor discharge, although not to the hypotension which also followed the injection of SP, and it was noted that the biggest increases in discharge were obtained in female cats.

Data obtained from five experiments in which chemoreceptor discharge, expressed as a percentage of the control spontaneous frequency, was pooled and plotted against time after an I.A. injection of SP are shown in Fig. 2. This quantitative evidence confirmed that higher doses of SP caused a delayed increase in chemoreceptor discharge, albeit a slight and somewhat variable effect. The duration of the increase, but not necessarily the peak discharge, appeared to be dose-related.

SP also caused a fall in arterial blood pressure which lasted for 1–5 min and was usually, but not invariably, polyphasic. The duration of the hypotension (i.e. time taken to return to control B.P.) was generally dose-related although the magnitude of the peak fall in pressure was not always related to the dose of SP (see Fig. 1). The increase in chemoreceptor discharge associated with higher doses of SP coincided with the onset of hypotension. However, although low doses of SP often caused B.P. falls similar to those evoked by much higher doses (see also Vogler, Haefely, Hürlimann, Studer, Lergier, Strässle & Berneis, 1963), there was often very little or no increase in discharge, and sometimes an increase in discharge occurred which was accompanied by a *rise* in B.P. (Figs. 3 and 4). These results suggested that the increase in chemoreceptor activity was not necessarily secondary to the hypotension caused by SP, and this was confirmed by showing that chemoreceptor discharge still increased when the fall in B.P. was prevented by infusing dextran after the injection of SP (Fig. 4), or in cases where mean B.P. rose rather than fell.

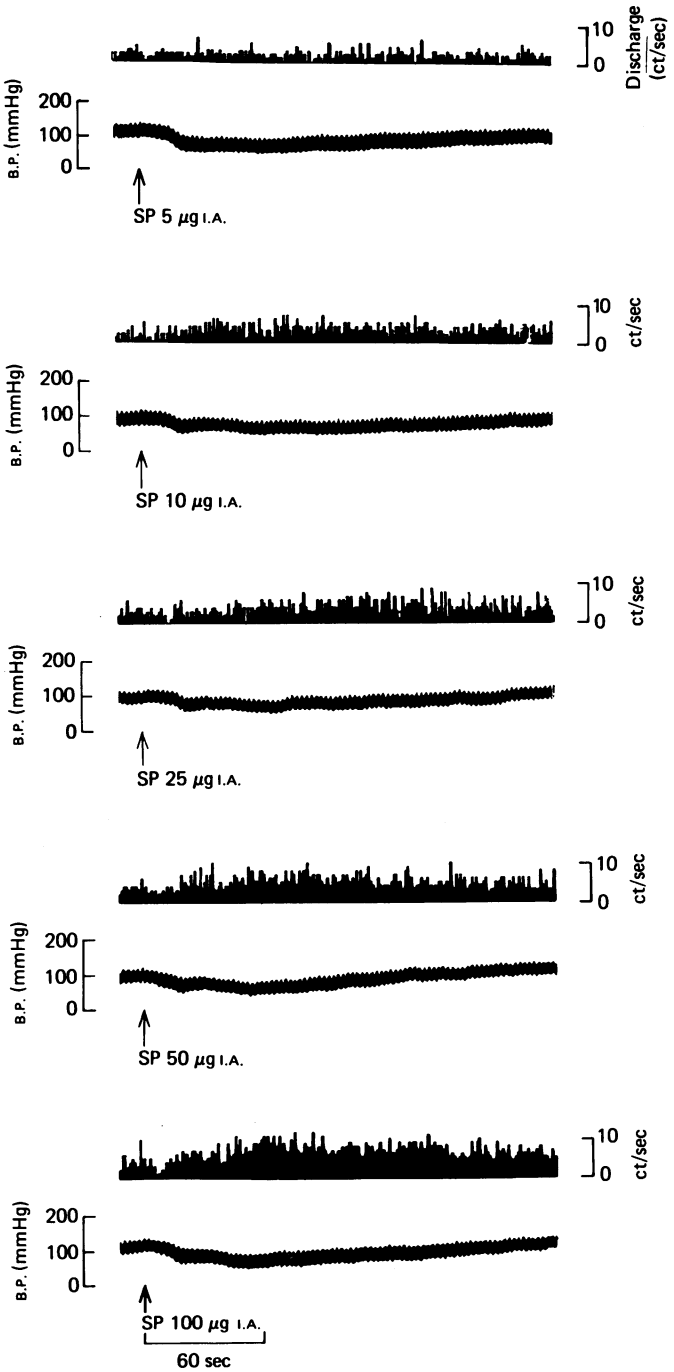


Fig. 1. Response of a single chemoreceptor unit to various doses of SP injected I.A. in random order with at least 20 min between successive doses. Each panel shows a block diagram of the chemoreceptor discharge in ct/sec and femoral arterial blood pressure. It can be seen in this particular experiment that although a similar fall in B.P. was evoked by all the doses of SP, the delayed increase in chemoreceptor discharge was dose-dependent.

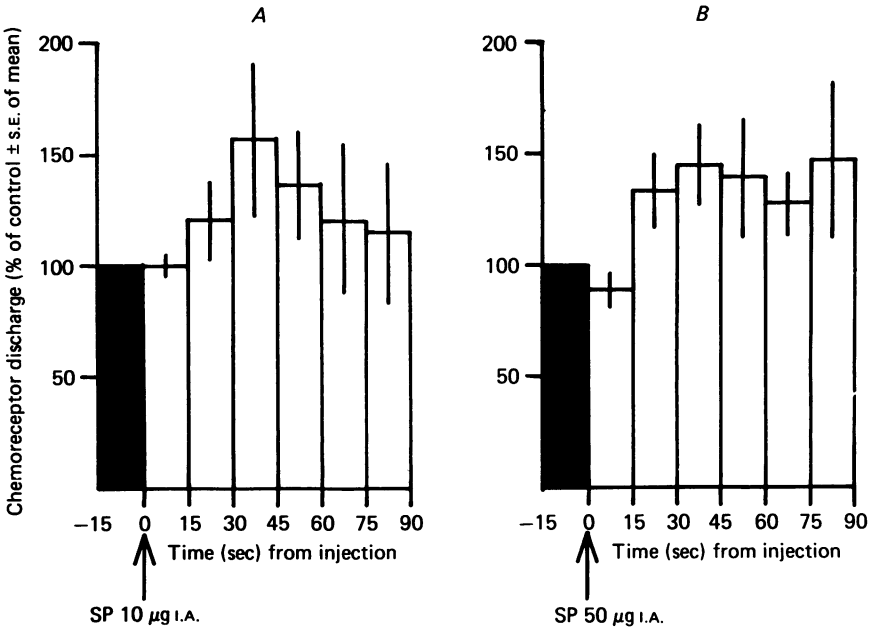


Fig. 2. Block diagrams showing chemoreceptor discharge following I.A. injections of 10 μg SP (*A*) and 50 μg SP (*B*). Discharge was averaged over 15 sec periods and expressed as a percentage of the average discharge in the 15 sec control period before the injection (black rectangle). Data obtained in different experiments were pooled and are shown as the mean percentage ± S.E. of the mean. The over-all average discharge in the control period was 2·8 ± 0·9 ct/sec for the four experiments with SP 10 μg I.A., and 2·5 ± 0·8 ct/sec for the five experiments with SP 50 μg I.A.

Bronchial and respiratory changes

In some experiments end-tidal CO_2 increased slightly at the time when spontaneous chemoreceptor discharge increased following SP I.A. This was reminiscent of the delayed (bigger) increase in end-tidal CO_2 seen following I.A. methacholine, an effect which was attributed to bronchoconstriction (McQueen, 1978*b*). It was necessary, therefore, to investigate whether the delayed increase in chemoreceptor discharge observed in the present experiments was secondary to changes in blood gas tensions resulting from a bronchoconstrictor action of SP; it is known that SP can cause bronchoconstriction in guinea-pig lung (Bisset & Lewis, 1962; Bhoola, Collier, Schachter & Shorley, 1962).

If bronchoconstriction were responsible for the delayed increase in discharge, one would not expect to see the effect in a spontaneously breathing animal because as soon as gas tensions began to change as a result of bronchoconstriction, the central and remaining peripheral chemoreceptors would reflexly adjust ventilation. Recordings of chemoreceptor activity were obtained in three experiments and responses to SP obtained with the animals breathing spontaneously and again when they were artificially ventilated and paralysed. The results obtained are illustrated in Figs. 3 and 4. It was found that the increase in chemoreceptor discharge occurred during spontaneous respiration, although the effect was not so sustained

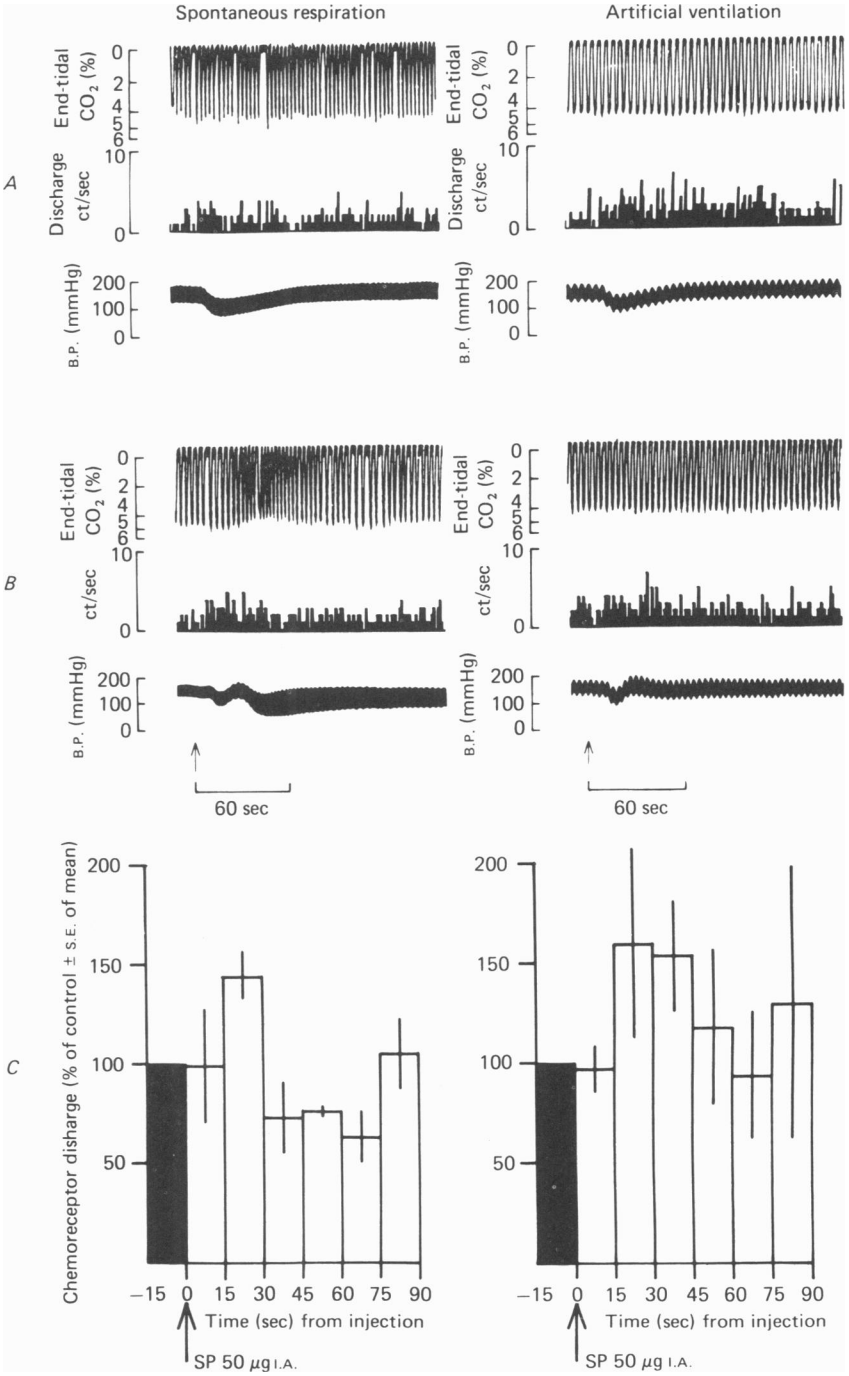


Fig. 3. Chemoreceptor discharge evoked by SP during spontaneous respiration compared with the effect observed on the same recording under conditions of artificial ventilation. *A* is the chemoreceptor discharge (three unit recording) in response to SP 1 μg I.A. *B* is from a different animal (two unit recording) showing the effects of SP 50 μg I.A. *C* presents the pooled data from three experiments in which SP 50 μg I.A. was injected at time 0, block diagram details being the same as for Fig. 2. The average control discharge was 1·3 ± 0·4 ct/sec during spontaneous breathing and 2·0 ± 0·8 ct/sec during artificial ventilation. The panels in *A* and *B* show, from above downwards. end-tidal CO_2; block diagram of chemoreceptor discharge in ct/sec; femoral arterial B.P. Injections represented by the arrows.

as that seen following the I.A. injection of SP when the animals were artificially ventilated (see Figs. 3 and 4). Arterial blood samples taken 45 sec after the injection of SP showed a fall in P_{a,O_2} and a rise in P_{a,CO_2} in artificially ventilated cats, but increases in P_{a,O_2} and slight decreases in P_{a,CO_2}, or no changes at all, when the animals were breathing spontaneously (see Fig. 4). The changes in gas tensions seen under conditions of artificial respiration were only slight (1–3 torr increase in P_{a,CO_2}; 1–5 torr decrease in P_{a,O_2}) but, because they interact (see Fig. 3 in Eyzaguirre & Lewin, 1961*b*), are probably responsible for some of the more *delayed* increases in chemoreceptor discharge.

Increases in chemoreceptor discharge were obtained when the animals were breathing spontaneously, despite the increase in respiration which occurred, even after the lowest dose of SP studied (0·1 μg I.A.). When the hypotensive action of SP was prevented by infusing dextran I.V. (see Fig. 4), there was no longer any marked change in respiration, thereby indicating that the increase in respiration was secondary to the hypotension.

Influence of SP injections on responses to ACh, NaCN, dopamine and hypoxia

During the study of the effects of SP on chemoreceptor discharge, responses to ACh (50 μg I.A.) and NaCN (5 μg I.A.) were determined before and 10–20 min after injecting SP in three experiments on artificially ventilated paralysed cats. Results obtained are shown in Fig. 5*A* and it was found that responses to NaCN were potentiated following SP, whereas those to ACh were slightly reduced. Inhibition evoked by dopamine (5 μg I.A.) was unaltered after SP. In two experiments SP (10 μg I.A.) was injected at the peak of the response to hypoxia (animal breathing 5 % O_2/95 % N_2) and had no effect on discharge. Although the results showed a potentiation of the response to NaCN, they were difficult to interpret because it was not known whether SP was directly influencing the chemoreceptors 10–20 min after it had been injected.

Infusions of SP

It was suspected that tachyphylaxis develops to the effects of SP on the chemoreceptors, so only short infusions of SP were studied in artificially ventilated paralysed cats. Since it was impossible to obtain dose–response data from such short infusions, single submaximal doses of chemoreceptor stimulants were used and responses obtained before and during a 5 min infusion of SP (1–50 μg/min) into one of the carotid catheters (see Methods). Sodium cyanide was injected into the other catheter 2 min after the infusion began, and ACh injected 4·5 min into the infusion. A fall in B.P. occurred and was usually sustained for most of the infusion period.

Results from three experiments are summarized in Fig. 5*C*. There was a dose-dependent potentiation of responses to NaCN and an inhibition of responses to ACh during the infusion of higher doses of SP. Responses to ACh and NaCN were largely unaltered when the drugs were injected during an infusion of 1 μg SP/min, or during the infusion of Locke solution. Spontaneous discharge was affected variably during the SP infusions, there being no obvious trend (see Fig. 5).

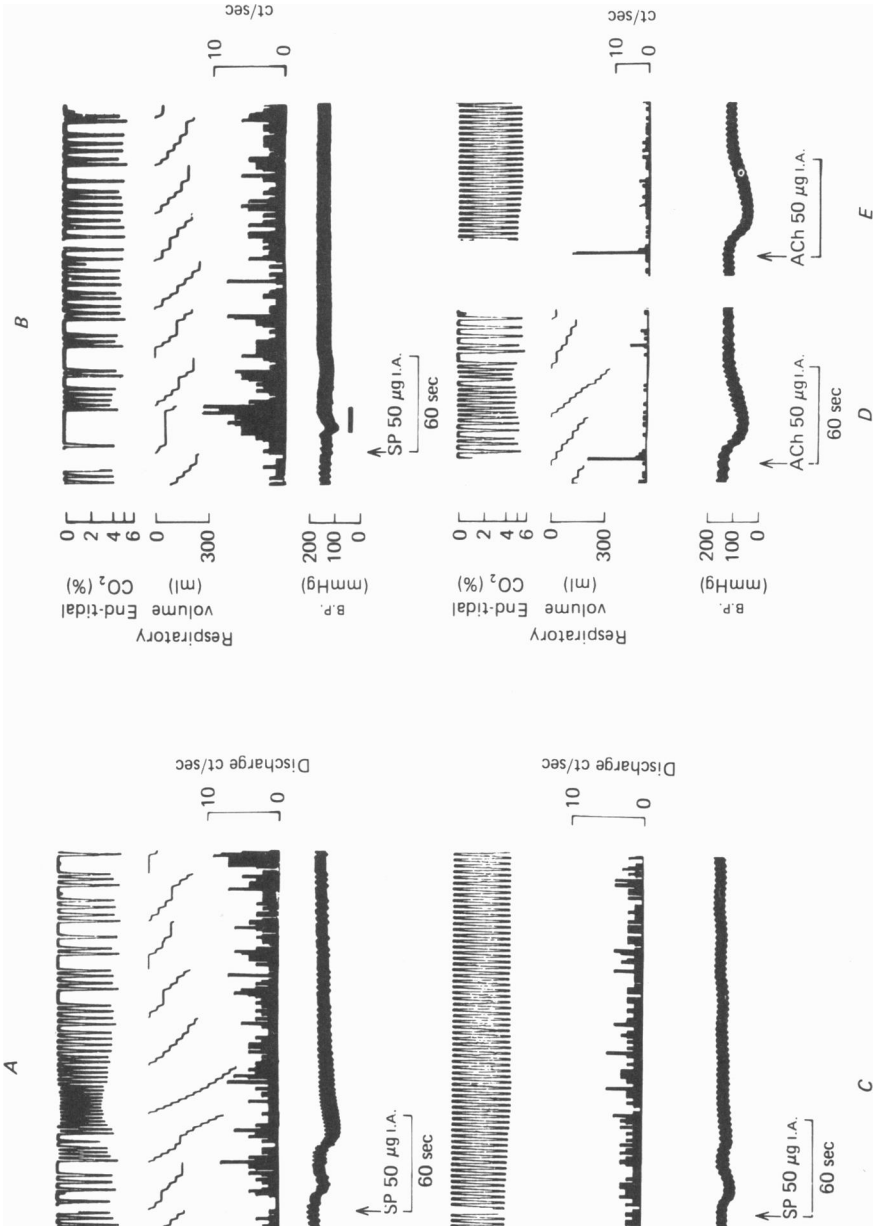


Fig. 4. Responses obtained during an experiment showing the effects on chemoreceptor discharge (three unit recording) of: *A*, SP 50 *μg* I.A. with the animal breathing spontaneously; *B*, SP 50 *μg* I.A. with the animal breathing spontaneously and dextran solution infused I.V. during the period represented by the horizontal black bar to maintain mean B.P.; *C*, SP 50 *μg* I.A. with the animal artificially ventilated. Responses to ACh 50 *μg* I.A. were also obtained during spontaneous breathing (*D*) and artificial ventilation (*E*).

Panels show from above downwards: end-tidal CO_2, the trace being interrupted at certain points because of the need to record voice on the same channel of the tape recorder; breath-by-breath record of respiration, total height representing the respiratory volume in 30 sec (only shown when the animal was breathing spontaneously); block diagram of chemoreceptor discharge in ct/sec; femoral B.P. Arterial blood samples were taken just before and also 45 sec after the SP injections. Readings (P_{a,CO_2}, P_{a,O_2} mmHg) were *A*: 33, 87 before, and 30, 100 after SP; *B*: 30, 97 before, and 30, 94 after SP; *C*: 31, 105 before, and 33, 100 after SP 50 *μg* I.A.

Shorter infusions of SP

These experiments were performed in order to avoid some of the problems associated with longer infusions (e.g. the risk of tachyphylaxis; sustained changes in B.P. or blood gas tensions leading to alterations in chemoreceptor sensitivity). Responses to ACh, NaCN, dopamine, and 5-HT were examined individually during short (60 sec) I.A. infusions of SP, the injection being made 45 sec after the start of the SP infusion. The order in which the different concentrations of SP were infused was varied randomly from experiment to experiment, and infusion of the same volume of Locke solution (0·5 ml. over 1 min) had no effect on the responses.

Results obtained from three experiments are summarized in Fig. 5*B*. During these short infusions there was a dose-related increase in responses evoked by NaCN, an effect which although less intense, is similar to that seen during the longer infusions. Responses to ACh were reduced, slightly during infusion of 1 μgSP/min and more markedly during 50 μg/min, this being similar to the results obtained during the longer infusions. However, responses to ACh obtained during 10 μgSP/min, although somewhat variable, were potentiated, whereas they were inhibited during the longer infusion.

The inhibiting response evoked by dopamine (5 μg I.A.) was reduced during SP 1 μg/min (to 52 ± 19 % of the pre-infusion or control response, $n = 3$; background (control) = $3{\cdot}6 \pm 2{\cdot}9$ ct/sec; during infusion = $1{\cdot}1 \pm 0{\cdot}7$ ct/sec) and SP 50 μg/min (to 64 ± 5 %, $n = 2$; background (control) = $1{\cdot}1 \pm 0{\cdot}5$, during infusion = $0{\cdot}4 \pm 0{\cdot}3$ ct/sec). During SP 10 μg/min there was a very variable effect with, over-all, a reduction of the inhibition (to 92 ± 26 %, $n = 3$; background (control) = $1{\cdot}2 \pm 0{\cdot}7$, during infusion $1{\cdot}0 \pm 0{\cdot}5$ ct/sec). The trend was, therefore, a reduction in the inhibitory response to dopamine during SP infusions, an effect which was not obviously dose-related. It should be noted that background activity decreased slightly during the SP infusions.

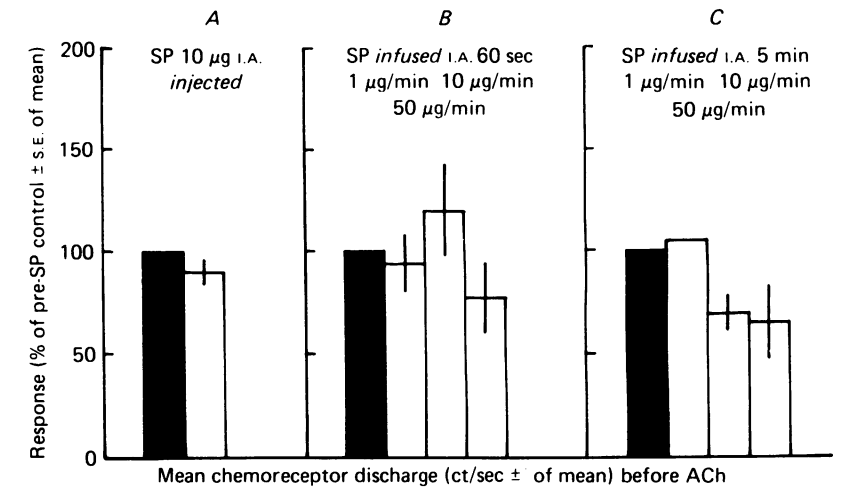
The brief stimulation of chemoreceptor activity evoked by 5-HT (10 μg I.A.) was reduced during SP infusions of 1 and 10 μg/min in one experiment. The delayed inhibition which followed the initial excitation (see Docherty & McQueen, 1978) was, however, potentiated during both the 1 and 10 μg/min infusions, although background discharge was slightly decreased by the SP.

Spontaneous chemoreceptor discharge was affected variably during the infusions of SP, there being a slight tendency for it to be decreased (see Fig. 5 and the dopamine results).

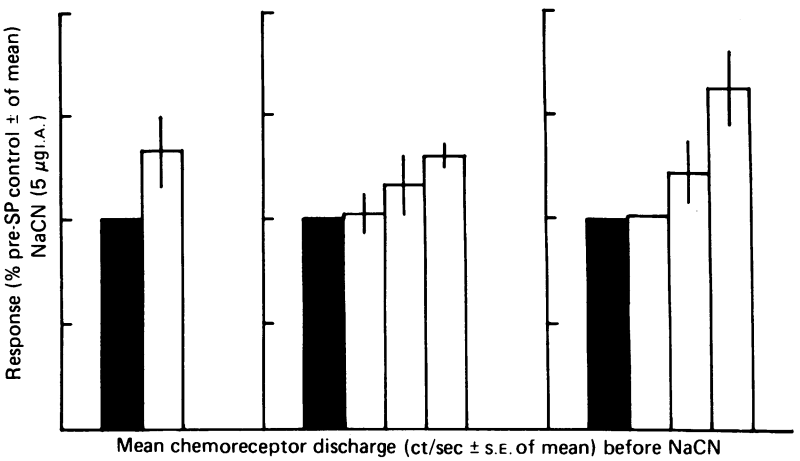
Injection and infusion of crude SP

A sample of Gaddum's SP was *injected* I.A. in an artificially ventilated cat which had not been paralysed. A slight fall in B.P. occurred and was followed by a rise in pressure. There was no immediate change in spontaneous chemoreceptor discharge, but after a delay of about 30 sec there was an increase which lasted for about 90 sec (see Fig. 6). Injection of the same amount of SP I.V. caused a slight fall in B.P. and also evoked a slight delayed increase in chemoreceptor discharge lasting from 40–120 sec after the injection.

During the *infusion* of crude SP (20 μg/min I.A. for 6 min) the response to NaCN



Before SP:	2·3 ± 0·7	2·1 ± 1·8	1·8 ± 0·9	1·3 ± 0·5	0·2	1·6 ± 0·9	1·5 ± 0·5
After or during SP:	3·0 ± 1·7	1·3 ± 0·9	1·3 ± 0·5	1·2 ± 0·3	0·2	2·8 ± 2·0	2·6 ± 1·4
n =	3	3	3	2	1	3	3



Before SP:	2·2 ± 0·7	1·6 ± 1·3	1·4 ± 0·7	1·2 ± 0·3	0·2	1·5 ± 0·5	1·9 ± 0·9
After or during SP:	3·4 ± 1·0	1·2 ± 0·9	0·8 ± 0·3	1·4 ± 0·6	0·1	2·6 ± 1·4	1·0 ± 0·6
n =	3	3	3	2	1	3	3

Fig. 5. Pooled data, obtained from the number of recordings indicated, showing the influence of SP on the response of the chemoreceptors to ACh 50 μg I.A. and NaCN 5 μg I.A. Responses are expressed as a percentage of the response ($\Delta\Sigma x$) obtained before SP was administered (black rectangle = control = 100 %). *A* shows the responses to ACh and NaCN injected 10–15 min after a single *injection* of SP (10 μg I.A.). *B* shows the effect on the responses evoked by the stimulants of a 60 sec *infusion* of SP at 1, 10 or 50 μg/min, the ACh or NaCN being injected 45 sec after the start of the infusion. *C* shows the responses evoked during a 5 min *infusion* of SP, NaCN being injected 2 min after the infusion started and ACh 4·5 min into the infusion. The average chemoreceptor discharge in the control periods is shown.

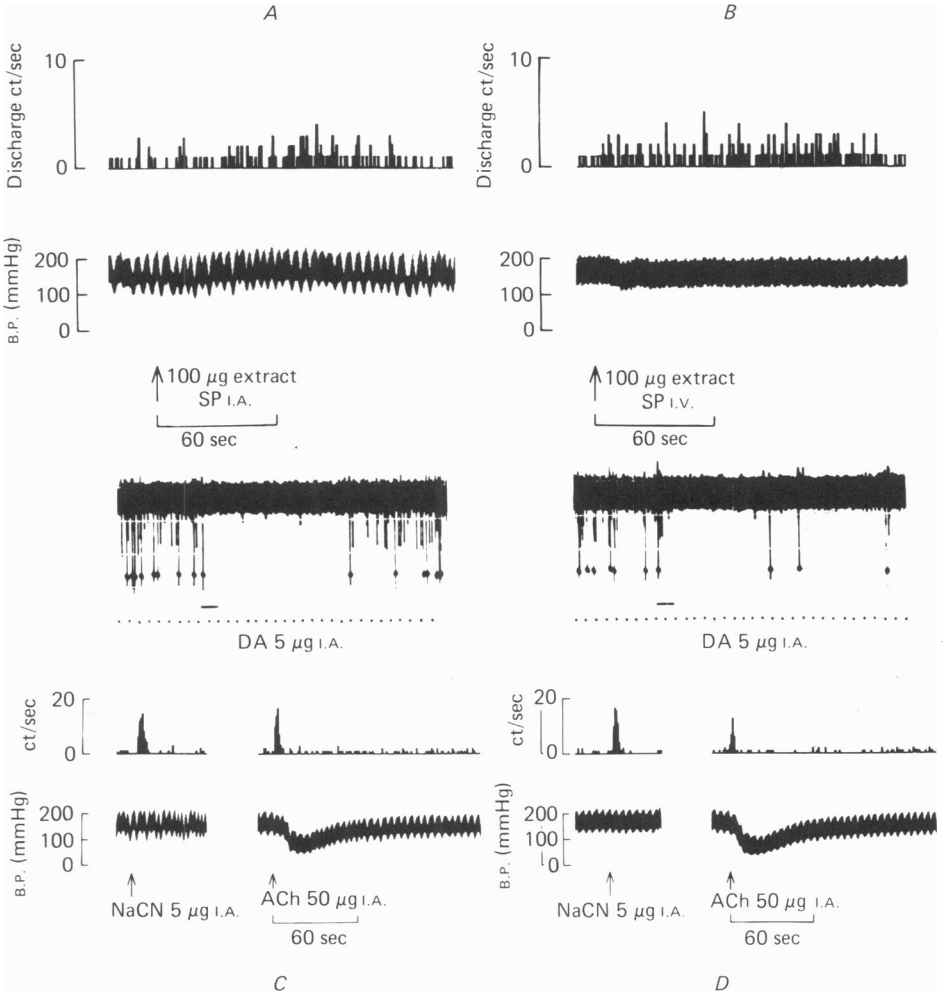


Fig. 6. Chemoreceptor activity (two units) from an experiment in which an extract of SP (1 mg extract ≏ 0·38 μg synthetic SP) was investigated. In *A* 100 μg extract was injected I.A. at the time indicated by the arrow, whereas in *B* the same dose was administered I.V. The animal was artificially ventilated but not paralysed. *C* shows responses to dopamine (DA) 5 μg, NaCN 5 μg and ACh 50 μg obtained during an infusion of Locke solution, whereas those in *D* are the responses obtained during a 6 min infusion of SP (20 μg extract/min). The neurograms show the action potentials with 1 sec timing marks below them, dopamine injections being represented by the horizontal bar. Action potentials were counted at the level represented by the brightening pulse. In the other panels, the upper trace is a bar diagram of chemoreceptor discharge (in ct/sec) with the lower trace showing B.P.

(injected at 3·5 min) was essentially similar to that obtained pre- and post-infusion (see Fig. 6). The response to ACh (injected at 5·5 min) was, however, reduced by about 30 % ($\Delta\Sigma x$) during the SP infusion, and there was a prolonged inhibition of spontaneous discharge following the initial excitation, an effect not observed before or after the infusion. The inhibitory action of dopamine (5 μg injected 1·5 min after the infusion started) also seemed to be potentiated during the infusion of crude SP.

DISCUSSION

Effect of SP on spontaneous chemoreceptor discharge

There was no evidence that exogenous SP caused any substantial change in spontaneous chemoreceptor discharge, such as is seen during the first 10 sec following an I.A. injection of ACh, NaCN or dopamine. However, about 10–20 sec after SP had been injected a long-lasting dose-dependent increase in discharge occurred. There were no consistent changes in spontaneous discharge during *infusions* of SP (1–50 μg/min I.A.), so it appears that the delayed increase is only obtained following the *injection* of high doses of SP. A classical extract of SP caused a delayed increase in discharge qualitatively similar to that evoked by synthetic SP.

Vascular effects. SP caused hypotension followed by a slight rise in B.P. (see also Pernow, 1953), and the present results confirmed that although the duration of the hypotension is dose-related (Vogler *et al.* 1963), it is often difficult to establish a clear dose–response relationship for the effect in cats (Burcher, Atterhög, Pernow & Rosell, 1977). The delayed onset of the increase in chemoreceptor discharge caused by SP and the fact that it tended to coincide with the fall in B.P. raised the question whether it was secondary to the hypotension. The sympathetic nerve supply to the carotid body had been cut, thereby eliminating the possibility that reflex changes in sympathetic activity, secondary to the hypotension, were responsible for increasing discharge by reducing blood flow through the glomus (Floyd & Neil, 1952; Biscoe & Purves, 1967). Chemoreceptor discharge is largely independent of B.P. over the physiological range (Hornbein, Griffo & Roos, 1961; Biscoe, Purves & Sampson, 1970; Acker, Keller, Lübbers, Bingham, Schulze & Caspers, 1973), as is demonstrated in the present experiments by the observation that although ACh, like SP, causes a fall in B.P., there was no marked increase in discharge associated with the hypotension (see Figs. 4 and 6).

The results showed that there was no correlation between the fall in B.P. and the delayed increase in discharge. In spontaneously breathing cats hypotension increased ventilation, an effect which is probably a consequence of several factors including changes in blood flow to the C.N.S. (Schmidt, 1928) and alterations in baroreceptor influences on bronchomotor tone (Daly & Schweitzer, 1951; Heymans & Neil, 1958; Widdicombe, 1963). Hyperventilation would tend to mask any delayed excitatory action of SP on the chemoreceptors. Although it is conceivable that SP could be influencing chemoreceptor discharge by altering the distribution of blood within the carotid body, the balance of evidence makes it unlikely that the delayed increase in chemoreceptor discharge is secondary to vascular effects of SP.

Bronchial effects. Evidence obtained from experiments on artificially ventilated cats showed that injected SP causes delayed changes in arterial blood gas tensions. This effect might have been the result of SP acting directly or indirectly in the lung to cause bronchoconstriction, although whether this was in fact the mechanism remains to be established. However, the increase in chemoreceptor activity, although not so sustained, still occurred in spontaneously breathing cats, even though the arterial blood gas tensions were either unaltered or changed in the opposite direction (P_{a,O_2} increased, P_{a,CO_2} decreased) as a consequence of hyperventilation secondary to the hypotension which followed the SP injection (see above). It appears, therefore,

that the increase in chemoreceptor discharge seen following an I.A. injection of SP can occur independently of respiratory or bronchial changes caused by SP, although such changes do modify the magnitude and duration of the increase. Since vascular and bronchial changes caused by SP are unable to account entirely for the increase in chemoreceptor discharge, particularly during the early phase of the response, and since some other potential secondary explanations can be precluded (e.g. SP does not release catecholamines from the cat adrenal medulla (Feldberg & Lewis, 1964; Lewis & Reit, 1966)), the effect could be due to a direct action of SP on the chemoreceptors.

Direct action of SP on chemoreceptors. It is fairly well established that SP can excite certain neurones in the C.N.S. (Otsuka, Konishi & Takahashi, 1975) and it has been hypothesized that SP may have a physiological role as a central neurotransmitter (Lembeck, 1953) or modulator of neural activity (Krivoy, Kroeger & Zimmermann, 1977). As far as the peripheral nervous system is concerned, Juan & Lembeck (1974) reported that synthetic SP excited peripheral sensory nerve endings associated with paravascular pain receptors in the rabbit ear. They noted a delay of '4–10 sec or more' before neural activity increased in their preparation and Krnjević (1977) also found a delay of 10–30 sec before an excitatory effect was observed following the ionophoretic application of SP to central neurones in cats. The latency and long time course of the effect has been attributed to the time taken for SP to diffuse through neural tissue (Otsuka & Konishi, 1977). However, the possibility that SP may be releasing another agent, or metabolized to an active entity, cannot be precluded. In the present experiments it was not possible to determine whether the chemoreceptors were uniformly affected by SP, or whether there was a difference between, for example, units associated with A fibres and those with C fibres. More recent evidence casts doubts on whether SP can, in fact, stimulate sensory nerve endings. Lembeck, Gamse & Juan (1977) found that synthetic SP was devoid of effect on paravascular pain receptors and were unable to explain the increase in activity obtained with synthetic SP by Juan & Lembeck (1974).

It is possible that SP was acting directly on the chemoreceptors to cause the increase in discharge. Any early excitatory action may have been masked by vascular effects of SP within the carotid body, or by an inhibitory action of SP, directly or indirectly mediated, on the chemoreceptors. However, whether the chemoreceptor-stimulating effect observed in the present experiments is due to primary or secondary actions of SP can only be resolved by further studies using a preparation, such as the *in vitro* carotid body (Eyzaguirre & Lewin, 1961*c*), which avoids many of the secondary complications.

Influence of SP on chemoreceptor responses to ACh, NaCN and dopamine

The results showed that the stimulant action of ACh was reduced after injections of SP and also during infusions of SP, with the exception of the unexplained potentiation during the short 10 μg/min infusion. In contrast, the excitant effect of NaCN was potentiated after injections of SP and, in a dose-related manner, during infusions of SP. Whatever effect SP was exerting on the chemoreceptors was evidently dose-dependent and fairly long-lasting: the response to NaCN was potentiated

10–15 min after the injection of SP (10 μg I.A.). Responses during a prolonged infusion of SP were slightly greater than those obtained during a short infusion, which provides evidence that tachyphylaxis to SP did not occur and also that changes in responsiveness during prolonged infusions were not secondary to sustained changes in B.P. or blood gas tensions evoked by SP.

SP has a nicotinic-blocking action, as has been shown by Ryall & Belcher (1977) on Renshaw cells and by Livett, Kozousek, Mizobe & Dean (1979) on cultured adrenal chromaffin cells. The present results are compatible with a slight nicotinic-blocking action of SP on the carotid chemoreceptors because such an effect would reduce the response to ACh and potentiate the action of cyanide (McQueen, 1977). Further experiments are required to determine whether this is, in fact, the mechanism of action of SP on the chemoreceptors.

The present results confirm previous reports that dopamine inhibits spontaneous chemosensory discharge as does 5-HT, after a brief initial excitation (e.g. see Docherty & McQueen, 1978). The inhibition evoked by dopamine was reduced during SP infusions whereas that associated with 5-HT was potentiated. It was not possible to determine what was responsible for these effects, nor what effect SP was having on the release of dopamine or 5-HT, both of which are present in the carotid body (Chiocchio, Biscardi & Tramezzani, 1967; Chiocchio, King, Carballo & Angelacos, 1971). It has been shown in cats that intra-nigral SP results in an increased release of dopamine (Chéramy, Niedullon, Michelot & Glowinski, 1977) and, in rats, that SP injected into the lateral ventricles stimulates the synthesis and utilization of dopamine and 5-HT in brain (Carlsson, Magnusson, Fisher, Chang & Folkers, 1977). SP has also been shown to be capable of affecting the release of 5-HT in the rat substantia nigra (Reubi, Emson, Jessell & Iversen, 1978). It would seem worth investigating whether SP influences carotid body dopamine and 5-HT; the outcome of such studies might explain the present results with exogenous dopamine and 5-HT.

Physiological significance of the results

The finding that spontaneous chemoreceptor discharge is slightly increased following close-arterial injection of SP to the carotid body, and that SP may be causing a slight nicotinic-blocking action on the chemoreceptors, might be considered to be of pharmacological rather than physiological interest because high doses of SP were needed to show the effects. It has also to be borne in mind that exogenous synthetic SP may not be mimicking the effect of any endogenous SP-like material that may be present in the carotid body; vascular or other non-specific effects of the injected SP may mask the primary action, and effects on cell metabolism (e.g. affecting dopamine or 5-HT as discussed above), glomus blood vessels, or even as a hormone, may be of more importance than any direct effect on sensory nerve activity. In any event, the present experiments would only detect direct effects of exogenous SP on the chemosensory mechanism which show up within a few minutes, which may not be the appropriate time course for any physiological action. A specific SP antagonist would have been very useful in the present experiments, but unfortunately none exists; baclofen (Saito, Konishi & Otsuka, 1975) is not a specific SP antagonist (Carlsson *et al.* 1977; Krnjević, 1977).

The physiological significance of the results depends on whether or not SP is present in the cat carotid body. As mentioned in the Introduction, there is evidence that a polypeptide is present in the carotid body, and SP-like material has been detected in various peripheral sensory nerves; SP is probably released from nerve endings of small C fibres (Hökfelt *et al.* 1977). Recent immunohistochemical evidence shows that SP-like material is present in some 5-HT-containing neurones in the rat C.N.S. (Hökfelt, Ljungdahl, Steinbusch, Verhofstad, Nilsson, Brodin, Pernow & Goldstein, 1978) and, as mentioned above, there are large numbers of 5-HT-containing cells in the cat carotid body. But is there any SP in the carotid body? Hanbauer (1977) was unable to detect any in the rat carotid body using a sensitive radioimmunoassay technique, but whether or not there is any in the cat carotid body remains to be established.

The author gratefully acknowledges the financial support of the Medical Research Council and the technical assistance of Mrs S. M. Bond.

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