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Changes in carotid body amine levels and effects of dopamine on respiration in rats treated neonatally with capsaicin

D.S. McQueen & A.K. Mir[1]

Department of Pharmacology, University of Edinburgh Medical School, 1 George Square, Edinburgh 8 and Department of Pharmacology and Therapeutics, University of Leicester, University Road, Leicester 1

1 Dopamine levels in rat carotid bodies and the effects of intravenous dopamine injections on respiration in adult rats anaesthetized with pentobarbitone have been studied in animals which were treated with capsaicin neonatally.

2 Levels of dopamine were five fold higher in the carotid bodies of capsaicin-treated rats as compared with vehicle-treated controls, but there was no significant difference between capasicin-treated and vehicle-treated rats in their ID_{50} values for dopamine-induced respiratory depression.

3 Domperidone, a dopamine D_2-receptor antagonist, substantially reduced the respiratory depression caused by dopamine, both in capsaicin-treated and in control animals, suggesting that a D_2-receptor was involved in the response. Cutting the carotid sinus nerves greatly reduced the ventilatory-depressant effect of dopamine, showing that sensory receptors, most probably arterial chemoreceptors, were responsible for most of the response.

4 Substantially less reflex hyperventilation was evoked in capsaicin-treated rats by the peripheral chemoreceptor stimulants hypoxia and sodium cyanide, in comparison with the controls, and domperidone did not increase the responsiveness. About 80% of the reflex ventilatory change originated from carotid body chemoreceptors.

5 The hypoventilation caused by breathing 100% O_2 was not significantly different in capsaicin-treated rats when compared with controls. Domperidone substantially reduced this response in capsaicin-treated rats, but not in vehicle-treated animals.

6 Dopamine-induced respiratory depression in capsaicin-treated rats was slightly enhanced, rather than reduced, by oxygen breathing; domperidone remained an effective antagonist of dopamine-induced ventilatory depression.

7 Most of the reduction in respiration caused by dopamine in rats anaesthetized with pentobarbitone can be attributed to actions on a dopamine D_2-receptor located in the carotid body. However, despite the increased levels of dopamine found in the carotid bodies, the reduced peripheral chemosensitivity observed in anaesthetized capsaicin-treated rats does not appear to result from a change in sensitivity to dopamine.

Introduction

Reflex increases in respiration evoked by stimuli that activate peripheral arterial chemoreceptors are significantly reduced in anaesthetized adult rats which have been treated neonatally with capsaicin (Bond *et al.*, 1982), but the cause of this change in sensitivity remains to be established.

Dopamine depresses respiration in anaesthetized rats apparently by inhibiting arterial chemoreceptors (Hasan & Horn, 1980; Horn, 1981; Cardenas & Zapata, 1981; Horn *et al.*, 1984; Zapata *et al.*, 1984) and dopamine is present in rat carotid bodies and can be released from them during stimulation (Hellstrom, 1977; Hanbauer & Hellstrom, 1978). It seemed possible, therefore, that excessive dopamine release, or increased sensitivity of chemoreceptors to dopamine, might explain the reduction in peripheral chemosensitivity caused by capsaicin treatment. In

[1] Present address: Merrell Dow Research Institute, 16 rue d'Ankara, 67084 Strasbourg, France.

order to test this possibility two separate series of experiments were performed. Firstly, biochemical techniques were used to measure amine levels in carotid bodies taken from capsaicin-treated and from control rats. Secondly, a series of experiments was performed which involved recording ventilation in anaesthetized rats. The respiratory responsiveness to dopamine and domperidone, a dopamine D_2-receptor (see Kebabian & Calne, 1979) antagonist that blocks dopamine-induced chemodepression in cats and rabbits (McQueen, 1984; Mir *et al.*, 1984), of capsaicin-treated animals was compared with that of vehicle-treated controls. The sensitivity of the peripheral chemoreceptors to physiological and pharmacological stimuli was also tested in both groups.

Methods

Experiments were performed on Sprague-Dawley rats. Animals were anaesthetized with halothane (1% in O_2) on day 2–4 after birth and injected with either capsaicin (50 mg kg^{-1} s.c.) or drug vehicle (10% ethanol, 10% Tween 80 in saline) as previously described (Jancso *et al.*, 1977; Bond *et al.*, 1982). Three to eight months later they were used for experiments.

Estimation of amine levels in rat carotid bodies and superior cervical ganglia

Rats were anaesthetized with pentobarbitone (42 mg kg^{-1} i.p.) and their carotid bodies and superior cervical ganglia excised surgically as previously described (Mir *et al.*, 1982). Carotid bodies and ganglia were homogenized in 300 μl and 1000 μl respectively of 0.1 M ice-cold perchloric acid. The content of dopamine, noradrenaline and 5-hydroxytryptamine (5-HT) was assayed using high performance liquid chromatography (h.p.l.c.) coupled to electrochemical detection, as previously described (Mir *et al.*, 1982) except that a reverse phase/ion pair 5 μ ultrasphere column was used for amine separation.

Respiratory experiments

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p., supplemented as required during the later part of some experiments). The level of anaesthesia, assessed qualitatively, was similar in the two groups but capsaicin-treated rats sometimes required a brief period (5–20 min) of artificial ventilation to keep them alive during the 30–60 min after injecting pentobarbitone. The trachea was cannulated as were both femoral arteries (one for measuring BP, the other for taking 300 μl arterial blood samples) and a femoral vein (for drug administration). During some of the experiments the carotid sinus nerves were identified and cut at their junction with the glossopharyngeal nerves and both vago-sympathetic nerves were cut in the neck.

Ventilation was measured with an integrating pneumotachograph, as previously described (McQueen, 1973). The animals breathed either room air, 100% O_2, or a hypoxic gas mixture (10% O_2: 90% N_2). The respiratory response to a hypoxic stimulus was obtained by switching from air to 10% O_2 for 3 min and taking an arterial blood sample at 2.5 min. Ventilation was measured 1 and 3 min after changing the inspired gas, and the same protocol was used for hyperoxia (100% O_2 inspired).

Drug administration

Doses of dopamine or sodium cyanide were injected intravenously (0.1 ml drug solution washed in with 0.2 ml Locke solution over 2–3s) with at least 5 min between successive doses. The respiratory volume (R.M.V.) during the 20s period (2 × 10 s ramps) immediately following the injection was calculated and expressed as a percentage change from the pre-injection (control) value and $\log_{10}$ dose-response lines plotted. Mean arterial BP was measured before and during the response period.

Statistical analysis

Mean values are given ± s.e. mean. Differences between groups were compared by using either the Wilcoxon two-sample test, the Wilcoxon signed ranks test (for paired data) or Student's paired *t* test (when there was insufficient data for the non-parametric test) and the null hypothesis rejected at P (2 tailed) $\leqslant 0.05$.

Drugs

Capsaicin (Sigma) was prepared as described above. Other drugs were dissolved in modified Locke solution (Docherty & McQueen, 1978): dopamine HCl (Koch-Light); sodium cyanide (B.D.H.); domperidone (kindly donated by Janssen Pharmaceuticals, Belgium).

Results

Amine measurements

Carotid bodies were excised from five capsaicin-treated rats (1 male 450 g; 4 females 256 ± 9.6 g) and from vehicle-treated controls (1 male 422 g; 4 female 249 ± 9.0 g) when the animals were four months old.

The amine levels in their carotid bodies, as estimated by h.p.l.c., are shown in Figure 1 and it was found that dopamine, noradrenaline and 5-HT levels were all significantly higher in capsaicin-treated rats compared with the controls ($P<0.05$). There was no significant difference between amine content of left and right carotid bodies within either group.

Superior cervical ganglia (SCG) from capsaicin-treated rats also had significantly higher dopamine levels (215 ± 8.5 pmol per SCG, $n=9$, $P<0.05$) than the controls (60.3 ± 1.7 pmol per SCG, $n=7$), but noradrenaline levels in capsaicin-treated animals (142 ± 2.3 pmol per SCG, $n=9$) were not significantly different from controls (142 ± 12 pmol per SCG, $n=7$, $P>0.05$).

Respiratory responses to dopamine

Dopamine (5.3–105 nmol) caused a dose-dependent depression of ventilation (V) when injected i.v. in capsaicin-treated and in vehicle-treated rats (Figure 2). The maximum reduction that could be obtained during the 20 s post-injection period varied from animal to animal, but averaged $55\pm4\%$ in vehicle-treated rats and $63\pm4\%$ in the capsaicin-treated group ($P>0.05$). The dose causing half-maximal depression of ventilation (ID_{50}) was calculated for each experiment (e.g. Figure 3) and averaged

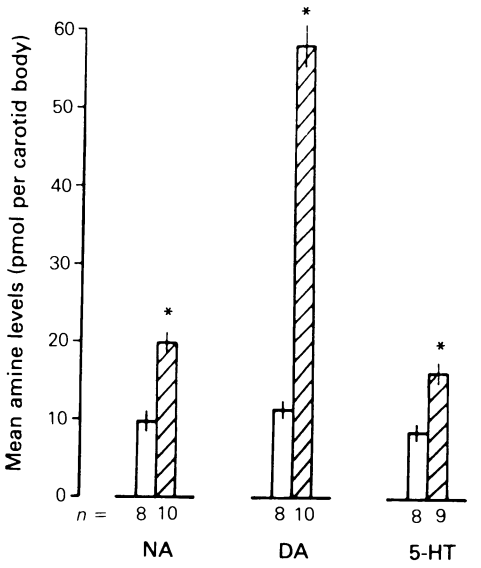

Figure 1 Amine levels, estimated by h.p.l.c., in carotid bodies of vehicle treated (open columns) and capsaicin-treated rats (hatched columns). Vertical lines show s.e.mean. n = number of carotid bodies. *$P<0.01$ (Wilcoxon two sample test). NA = noradrenaline, DA = dopamine; 5-HT = 5-hydroxtryptamine.

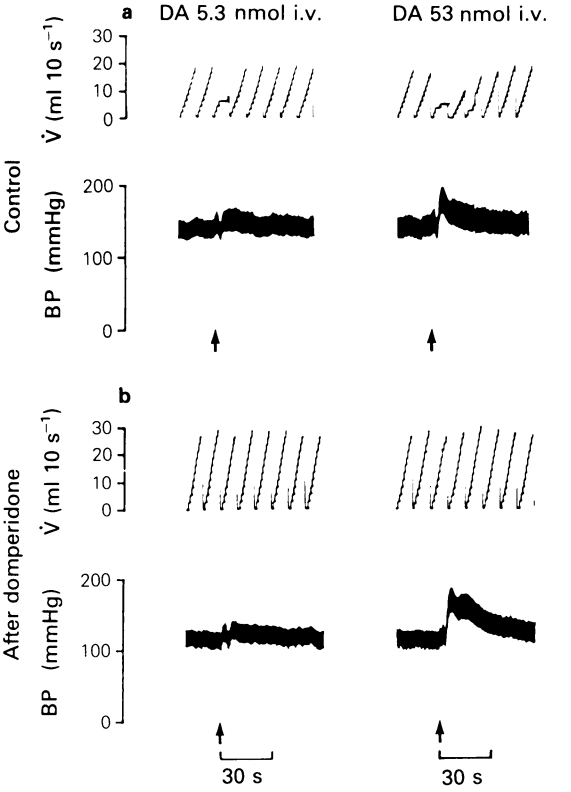

Figure 2 Respiratory (V) and blood pressure (BP) responses to two doses of dopamine (DA; 5.3 and 53 nmol i.v. at arrows) in an anaesthetized capsaicin-treated rat (a) before and (b) after administering the dopamine D_2- receptor antagonist domperidone ($100\ \mu g\ kg^{-1}$ i.v.). The respiratory trace reads from bottom to top with each step representing a single breath and the total ramp height showing the volume breathed in 10 s. Note that domperidone substantially reduced the hypoventilation caused by dopamine, but not the rise in blood pressure.

74 ± 14 nmol kg^{-1} (range 26–141, $n=8$) in controls and 108 ± 24 nmol kg^{-1} (range 28–287, $n=13$) in capsaicin-treated rats. Average ventilation pre-injection was 30.6 ± 2.4 ml $100\,g^{-1}$ min^{-1} in the former and 32.0 ± 2.3 ml $100\,g^{-1}$ min^{-1} in the latter group. None of these values was significantly different between groups. Blood pressure changes were minimal with lower doses of dopamine, but higher doses caused a dose-related hypentesion (e.g. Figure 2). The mean rise in BP associated with the 26 nmol dose of dopamine was 7 ± 5.5 mmHg in controls ($n=8$) and 12 ± 3.6 mmHg in capsaicin-treated rats ($n=13$; $P>0.05$).

The dopamine antagonist domperidone ($100\ \mu g\ kg^{-1}$ single dose) greatly reduced the respirat-

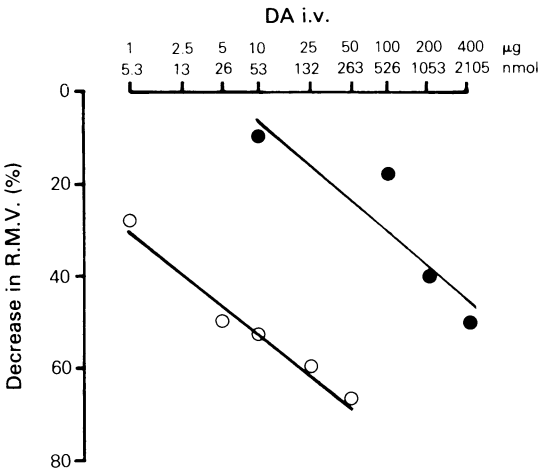

Figure 3 Dose-response plot of the respiratory responses to dopamine (DA) obtained during the experiment shown in Figure 2 before (○——○) and after (●——●) domperidone (100 μg kg^{-1} i.v.) in a 286 g capsaicin-treated female rat. Straight lines were fitted to the data by the least squares method and the dose required for half-maximal response (ID_{50}) calculated. The ID_{50} was 28 nmol kg^{-1} before domperidone, and 2611 nmol kg^{-1} for the same response after the antagonist. Mean ventilation (R.M.V.) averaged 39 ± 1.6 ml 100 g^{-1} min^{-1} pre-domperidone and 59.4 ± 1.7 ml 100 g^{-1} min^{-1} post-domperidone.

ory depressant effect of dopamine (see Figures 2 and 3) and the corresponding ID_{50} values obtained after the antagonist were 2300 ± 660 nmol kg^{-1} for controls ($n = 7$) and 3350 ± 990 nmol kg^{-1} for capsaicin-treated rats ($n = 11$). The ID_{50} values were significantly higher after domperidone but the difference between groups was not significant. Steady-state ventilation was 31.7 ± 2.5 ml 100 g^{-1} min^{-1} in control rats before domperidone and 37.8 ± 5.1 ml 100 g^{-1} min^{-1} after domperidone ($n = 7$; $P > 0.05$).

capsaicin-treated rats ventilation increased significantly from 32.6 ± 2.7 ml 100 g^{-1} min^{-1} to 40.7 ± 3.0 ml 100 g^{-1} min^{-1} ($n = 11$, $P < 0.05$-Wilcoxon signed ranks test). Domperidone did not antagonize the pressor response to dopamine (e.g. see Figure 2).

Respiratory responses to hypoxia and hyperoxia

A reflex increase in ventilation occurred when rats breathed 10% O_2: 90% N_2 instead of air, and although the response was present in both groups, it was significantly greater in vehicle-treated rats (Figures 4 and 5). Measurement of the percentage increase in respiration 1 and 3 min after starting a 3 min period of hypoxia showed that hyperventilation was well-maintained in vehicle-treated rats, but not in the capsaicin-treated animals (Figure 5). Arterial blood gas tensions and pH values are shown in Table 1.

When 100% O_2 was breathed, respiration was reduced to about 40% of the value on air, and this decrease was not significantly different in the two groups.

Domperidone

Domperidone (100 μg kg^{-1} single dose i.v.) significantly reduced ($P < 0.05$; paired t test) the mean increase in ventilation evoked by 10% O_2 in controls (Figure 5) but although the response was also reduced in capsaicin-treated animals, with ventilation at 3 min now being depressed rather than increased, responses were variable and the differences from pre-domperidone mean values were not statistically significant ($P > 0.05$; paired t test).

In the presence of domperidone the reduction in respiration caused by breathing 100% O_2 was significantly attenuated in capsaicin-treated rats, but although not so well sustained in controls, the difference from pre-domperidone responses was not statistically significant.

Blood pressure values obtained before and during

Table 1 Mean arterial blood gas tensions and pH in rats breathing air, 10% O_2 or 100% O_2

		Vehicle-treated			*Capsaicin-treated*		
		Air	*10% O_2*	*100% O_2*	*Air*	*10% O_2*	*100% O_2*
Pa_{O_2} (KPa)	=	10.0 ± 0.35	5.87 ± 0.25	46.0 ± 3.2	9.70 ± 0.42	5.05 ± 0.35	28.1 ± 2.1*
Pa_{CO_2} (KPa)	=	4.67 ± 0.31	3.73 ± 0.47	6.80 ± 0.49	4.63 ± 0.17	3.47 ± 0.34	6.04 ± 0.55
pH	=	7.40 ± 0.02	7.47 ± 0.02	7.26 ± 0.04	7.39 ± 0.01	7.48 ± 0.03	7.28 ± 0.03
n	=	12	10	5	11	11	7

Samples were taken 1 min before and 2.5 min after switching from air to hypoxic or hyperoxic gas.
* $P < 0.05$ vs vehicle-treated. All other capsaicin-treated, not significantly different ($P > 0.05$) from corresponding vehicle-treated values.

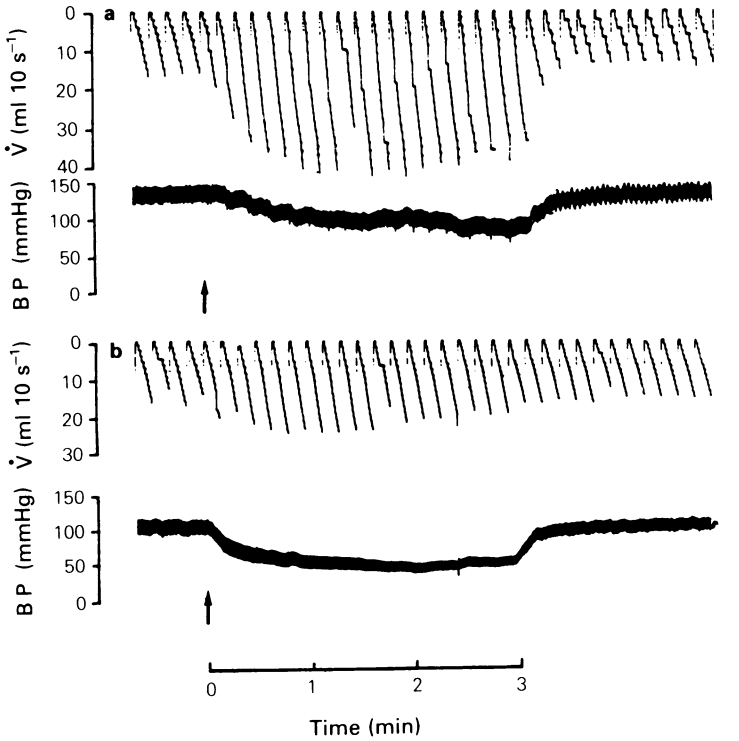

Figure 4 Respiratory and blood pressure responses to switching the inspired gas from room air to 10% O_2:90% N_2 for a 3 min period (commencing at arrow). (a) Vehicle-treated female rat (245 g). (b) Capsaicin-treated female rat (296 g) showing a much reduced and poorly sustained hyperventilation in response to the hypoxic stimulus as compared with the vehicle-treated control. The respiratory trace reads from top to bottom and interruptions at approximately 2 min intervals are caused by automatic amplifier resetting in the electrospirometer. Other details as in Figure 2.

hypoxia and hyperoxia, pre- and post-domperidone are shown in Figure 6. During 10% O_2 the mean BP in capsaicin-treated rats was significantly lower than in the vehicle- treated controls ($P<0.05$). After domperidone there was no statistically significant difference between groups. During oxygen breathing BP was significantly lower in capsaicin-treated rats compared with vehicle-treated controls ($P<0.05$). There was no significant difference in B.P. values pre- and post-domperidone measured during oxygen breathing in capsaicin-treated rats, but there was a small but significant difference ($P<0.05$, paired t test) between pre- and post-domperidone values from vehicle-treated animals.

Respiratory responses to sodium cyanide

NaCN was injected at two dose-levels, 1 and 4.1 μmol (50–200 μg) i.v. which produced responses that were suprathreshold but submaximal. Reflex hyperventilation was significantly greater in vehicle-treated controls than in capsaicin-treated rats (Figure 7). Domperidone reduced the ventilatory response to the high but not the low dose of cyanide in capsaicin-treated rats ($P<0.05$, paired t test), but in vehicle-treated rats the responses to either dose of cyanide before domperidone were not significantly different from the corresponding responses obtained after the antagonist.

Denervation experiments

In order to assess the relative contribution of different regions to the respiratory changes obtained, experiments were performed (mainly in vehicle-treated rats because capsaicin animals did not tolerate the procedure well) during which responses to dopamine, hypoxia, hyperoxia and cyanide were obtained before and after cutting the carotid sinus nerves. Subsequently, both vagosympathetic trunks were cut in the neck. In some experiments, the inverse sequence was followed, with the vagi being cut

first, and the carotid sinus nerves sectioned later in the experiment. Results obtained are summarized in Figure 8 which shows that cutting both sets of nerves totally abolished respiratory responses to hypoxia, cyanide and hyperoxia. Cutting the carotid sinus nerves, leaving the vagosympathetic trunk intact, reduced the ventilatory response to hypoxia by 83% (1st min), and the response was no longer well-sustained, ventilation being *depressed* by 20% at 3 min. Hyperoxia caused an increase in ventilation under these conditions, presumably reflecting removal of hypoxia-induced central respiratory depression, and the response to sodium cyanide was reduced by 80% compared with the intact preparation. Cutting the vagosympathetics abolished the residual responses to hypoxia and cyanide.

When the vagosympathetic trunks were cut first, leaving the carotid sinus nerve intact, the hyperventilation in response to hypoxia was only reduced by 16% and was well sustained. The response to hyperoxia was increased slightly and the response to cyanide reduced by 25% compared with the intact preparation. Cutting the carotid sinus nerves abolished the responses to hypoxia and sodium cyanide.

Cutting both sets of nerves substantially reduced the respiratory depressant action of dopamine obtained during air-breathing in vehicle-treated rats and most of the reduction could be attributed to the carotid sinus nerves since bilateral vagotomy alone did not greatly affect the response to dopamine. The effect of cutting both sets of nerves was thus rather similar to that obtained by administering domperidone.

Physiological denervation of arterial chemoreceptors was also attempted by ventilating the animal with 100% O_2 until a steady ventilatory state was reached. Responses to dopamine were studied during oxygen breathing and the respiratory changes measured. In vehicle-treated animals dopamine caused a depression of ventilation during oxygen breathing, but this was not a dose-related phenomenon. Domperidone reduced this response to dopamine, as it did during air breathing. In capsaicin-treated rats the dose-

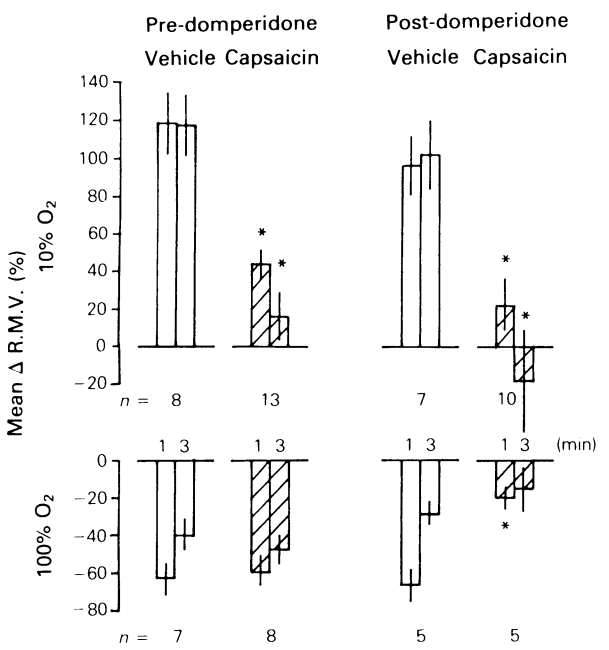

Figure 5 Mean changes in ventilation of vehicle-treated control (open columns) and capsaicin-treated (hatched columns) anaesthetized rats in response to switching from breathing room air to either 10% O_2 (upper panels) or 100% O_2 (lower panels) for 3 min Vertical lines show s.e. mean. Responses (1 and 3 min values shown for *n* animals) were obtained before and after injecting a single dose of domperidone (100 $\mu g\,kg^{-1}$ i.v.). $^*P<0.05$ in comparison with corresponding (i.e. pre- or post-domperidone) values from vehicle-treated rats using the Wilcoxon two sample test.

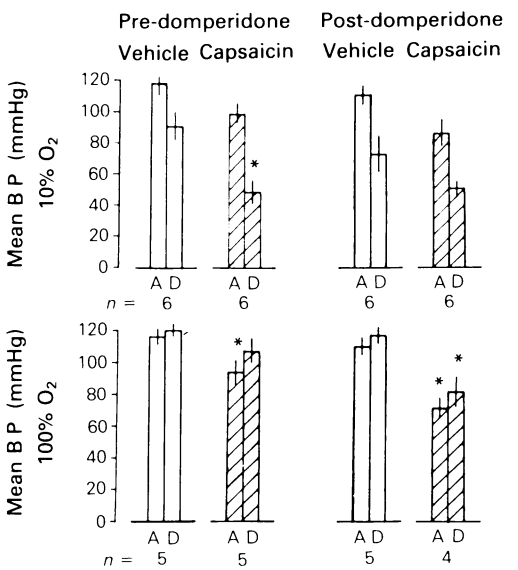

Figure 6 Mean arterial blood pressure of vehicle-treated (open columns) and capsaicin-treated (hatched columns) anaesthetized rats (A) during air breathing and during (D) either 10% O_2 (upper panels – at time of maximum change in BP) or 100% O_2 (lower panels) for 3 min before and after domperidone 100 $\mu g\,kg^{-1}$, i.v. Vertical lines show s.e. mean. $^*P<0.05$ in comparison with corresponding values (pre- and post-domperidone) for vehicle-treated animals using the Wilcoxon two sample test.

related respiratory depressant effect of dopamine was *potentiated* slightly by oxygen, and this effect was antagonized by domperidone. Dompamine-induced reductions in respiration obtained during oxygen breathing were greatly reduced by cutting the carotid sinus nerves, but not by cutting the vagosympathetic trunks in the neck.

Discussion

Our results are not compatible with the hypothesis that the reduced arterial chemosensitivity observed in anaesthetized adult rats which had been treated neonatally with capsaicin is caused by a change in the responsiveness of carotid chemoreceptors to dopamine (see Introduction). Although dopamine levels were five fold higher in the carotid bodies of capsaicin-treated rats in comparison with vehicle-treated controls, there was no significant difference between the two groups in the depression of respiration caused by intravenously injected dopamine, as judged from the ID_{50} values.

The respiratory data confirmed that the respiratory chemoreflex to stimulants such as hypoxia and sodium cyanide is significantly reduced in rats that had been treated neonatally with capsaicin (Bond *et al.*, 1982). Capsaicin mainly destroys unmyelinated sensory afferent fibres when injected neonatally in the doses used in this study (Jancso *et al.*, 1977) and capsaicin-treated rats have fewer unmyelinated fibres in the carotid sinus nerve as compared with vehicle-treated controls (see Bond *et al.*, 1982). Unmyelinated fibres account for 86% of the fibres in the rat sinus nerve (McDonald, 1983), although it is not known how many of these are chemosensory. The question of how destruction of sensory afferents by capsaicin is related to the increase in levels of dopamine, noradrenaline and 5-HT in the carotid body, and increased dopamine levels in the superior cervical ganglia, cannot be answered by the results from our study. It is also likely that other substances will be affected by capsaicin treatment, including substance P (Gamse *et al.*, 1980; Keeler & Black, 1981) which has been found in the rat carotid body (Jacobowitz & Helke, 1980) and might function as a neurotransmitter at the central terminals of afferent carotid sinus nerve fibres (Helke *et al.*, 1980).

The selective dopamine D_2-receptor antagonist domperidone (Baudry *et al.*, 1979; Lazareno & Nahorski, 1982), which is effective in antagonizing the chemodepressant action of dopamine in rabbit carotid bodies (Mir *et al.*, 1984), greatly reduced the hypoventilation caused by dopamine both in capsaicin-treated and vehicle-treated rats, the latter

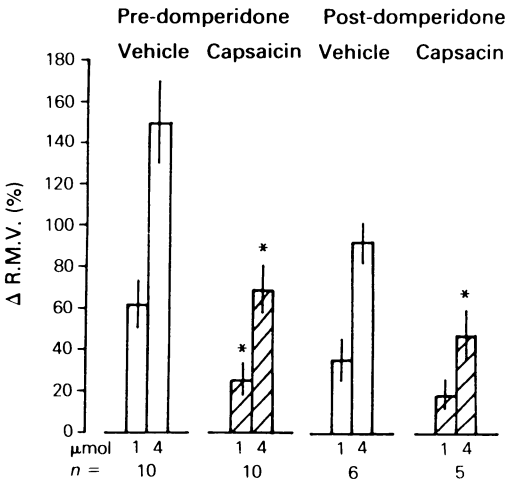

Figure 7 Increases in respiration evoked by two doses of cyanide (1 and 4 μmol i.v.) in *n* vehicle-treated control (open columns) and capsaicin-treated (hatched columns) anaesthetized rats before and after domperidone (100 μg kg^{-1} i.v.). Vertical lines show s.e. mean. Mean basal ventilation was 29.4 ± 2.1 in controls and 34.6 ± 2.9 ml 100 g^{-1} min^{-1} in capsaicin-treated rats before domperidone. Corresponding values post-domperidone were 37.7 ± 5.1 and 41.5 ± 3.5 ml 100 g^{-1} min^{-1}.* $P < 0.05$ in comparison with corresponding vehicle-treated controls (Wilcoxon two sample test).

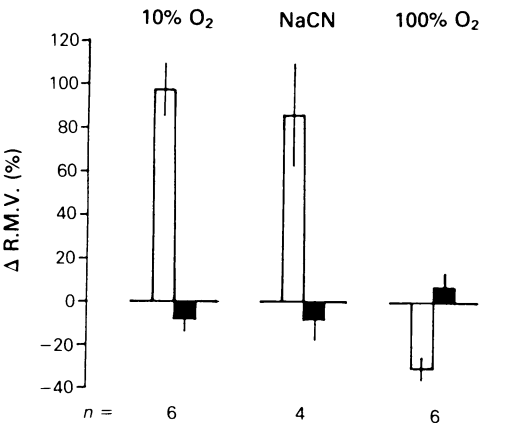

Figure 8 Ventilatory responses to breathing 10% O_2 or 100% O_2, both measured 1 min after switching from air, and to sodium cyanide 1 μmol i.v. during air breathing, were obtained before (open columns) and after (solid columns) cutting both the carotid sinus and vagus nerves in vehicle-treated rats. Vertical lines show s.e. mean. Ventilation in the steady state averaged 25.3 ± 3.1 ml 100 g^{-1} min^{-1} before and 22.9 ± 2.4 ml 100 g^{-1} min^{-1} after sectioning the nerves.

finding confirming the report by Horn *et al.* (1984). This evidence suggests that the respiratory-depressant effect of dopamine is mediated via a dopamine D_2-receptor, and similar conclusions were reached by Zapata *et al.* (1984) on the basis of respiratory experiments in rats using the dopamine antagonists sulpiride and metoclopramide. Since, in general terms, dopamine does not cross the blood brain barrier and neither does domperidone (Laduron & Leysen, 1979), it can be deduced that the respiratory depressant effect of DA must involve actions outside the CNS. The available evidence points to the carotid body as the major site at which dopamine acts to reduce respiration in rats (e.g. Horn *et al.*, 1984; Zapata *et al.*, 1984). Electrical recordings of chemosensory activity in the rat carotid sinus nerve (Cardenas & Zapata, 1981) have shown that, as in most species, dopamine depresses chemosensory discharge and would, therefore, be expected to cause hypoventilation by reducing peripheral chemoreceptor drive to respiration in rats anaesthetized with pentobarbitone (Horn *et al.*, 1984).

Cutting both carotid sinus nerves greatly reduced dopamine-induced ventilatory depression and also attenuated the reflex hyperpnoea caused by hypoxia or cyanide acting on the carotid chemoreceptors, and this evidence is consistent with an inhibitory action of dopamine on carotid body chemoreceptors. About 20% of the responses to hypoxia and cyanide survived bilateral cutting of the sinus nerves, but cutting both vagosympathetic nerves in the neck abolished the residual responses. These extra-carotid effects may have originated from thoracic or abdominal chemoreceptors (see Cardenas & Zapata, 1983) or perhaps from aortic chemoreceptors, although the latter have been found to be non-functional in rats (Sapru & Krieger, 1977a, b; 1978).

There were some differences from a previous study (Bond *et al.*, 1982), mainly in relation to the absolute values for R.M.V. during steady state conditions, and to the arterial blood gas tensions. These differences may, in part, be explained by the fact that in the present experiments the animals were more deeply anaesthetized than was the case previously. This was a deliberate policy in order to make the results more directly comparable with those of Horn *et al.* (1984) and Cardenas & Zapata (1981) who used a 60 mg kg^{-1} dose of pentobarbitone. Unexpected results were obtained when dopamine was injected into vehicle-treated rats which were breathing 100% O_2 under steady-state conditions. It was anticipated that the increased oxygen tension would virtually abolish peripheral chemosensory activity ('physiological denervation' of the chemoreceptors) and so prevent dopamine from depressing ventilation (i.e. there would be no respiratory drive from the chemoreceptors for dopamine to depress). In fact dopamine still caused some hypoventilation, but the responses were not dose-related. Similar, although less intense, effects can be seen in the results of Horn *et al.* (1984). The cause of this responsiveness to dopamine in rats breathing oxygen is difficult to ascertain, but since it was partially antagonized by domperidone it apparently involves actions at dopamine D_2-receptors. Rats anaesthetized with pentobarbitone and breathing oxygen may still have a significant peripheral chemoreceptor drive to respiration either originating from within the carotid body (perhaps involving CO_2) or due to enhanced gain in the CNS, and this possibility could be investigated by recording chemosensory activity from the sinus nerve.

Although capsaicin-treated rats show a reduced respiratory response to peripheral chemoreceptor stimulation, sudden withdrawal of chemosensory input as a consequence of switching to breathing 100% O_2 caused just as marked a hypoventilation in these animals as it did in the vehicle-treated controls. This implies that, although chemoreflex activity is reduced by capsaicin treatment, there is still sufficient chemoreceptor drive to respiration under the conditions of our experiments to give a normal response to oxygen-breathing. Domperidone had no significant effect on oxygen-induced hypoventilation in controls, but did significantly attenuate the response in capsaicin-treated rats. However, it is difficult to evaluate this evidence because systemic arterial blood pressure was significantly lower in capsaicin-treated rats during oxygen breathing after domperidone in comparison with vehicle- treated controls, which complicates the interpretation. Further studies on this aspect are needed.

Ventilatory responses to hypoxia and cyanide were not potentiated after domperidone, yet should have been if endogenous dopamine was tonically inhibiting the chemoreceptors, particularly in capsaicin-treated rats. In fact responses tended to be reduced after the antagonist, although ventilation in the steady state was increased, significantly so in the case of capsaicin-treated rats. Horn *et al.*, (1984) also found a reduced respiratory response to hypoxia following administration of the dopamine D_2-receptor antagonist sulpiride. Thus, domperidone increases ventilation under normoxic conditions in capsaicin-treated rats, which could be taken as evidence for a greater release of dopamine occurring in the carotid bodies of these animals as compared with the vehicle-treated controls. This assumes that exogenous dopamine has similar effects to endogenous, which need not be the case. In contrast, however, the fact that domperidone reduced the ventilatory response to hypoxia could be regarded as evidence for endogenous dopamine, acting via a dopamine D_2-receptor, being involved in chemoexcitation. This is reminiscent of the 'dual effect' described by Car-

denas & Zapata (1980) whereby dopamine reduced chemoreceptor responses to low doses of cyanide but potentiated the effects of higher doses. In our experiments, domperidone may, by blocking the actions of endogenous dopamine, potentiate ventilatory responses when dopamine release is low (i.e. during normoxia) but reduce the responses when release is high (during hypoxia). Whether or not this does occur remains to be established.

In conclusion, within the limitations imposed by working on whole animals where secondary changes can affect primary reflex responses, and the anaesthetic agent provides further complications, it seems unlikely that the increased levels of dopamine found in the carotid bodies of rats treated neonatally with capsaicin are responsible for the reduced peripheral arterial chemoreflex activity because: (a) dopamine-induced ventilatory depression was not significantly altered in capsaicin-treated animals, and (b) the dopamine antagonist domperidone did not increase the respiratory responses evoked by the chemoreceptor stimulants hypoxia and cyanide. Further studies, possibly in conscious animals, are needed to determine how neonatal treatment with the neurotoxin capsaicin causes a reduction in respiratory responsiveness to peripheral chemoreceptor stimulation. It will also be interesting to investigate the extent to which central and/or peripheral effects of capsaicin influence the actions and interactions of polypeptides such as substance P and the enkephalins, which are present in the carotid body and can co-exist with monoamines (Hansen *et al.*, 1982; Varndell *et al.*, 1982).

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