The effect of octreotide on breathing and the ventilatory response to CO_2 in conscious dogs

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The effect of octreotide on breathing and the ventilatory response to CO_2 in conscious dogs. K. Cao, R.R. Grunstein, K.Y. Ho, C.E. Sullivan. ©ERS Journals Ltd 1998.

ABSTRACT: It has been reported that injection of somatostatin into the brain-stem will lead to apnoea in animals. The aim of this study was to determine whether peripheral administration of octreotide, an analogue of somatostatin, could influence the control of breathing.

We measured the tidal volume, respiratory rate and ventilatory response to CO_2, before and after the intravenous injection of two dose levels of octreotide (0.1 mg and 0.5 mg) or saline in four conscious adult dogs.

Injection of octreotide altered the breathing pattern with a mean decrease in the respiratory frequency of 23% (p<0.05) and an increase in the tidal volume by 16% (p<0.05), resulting in no net change in ventilation. The normal value of the ventilatory response to CO_2 ranged between 1.0–3.2 $L \cdot min^{-1} \cdot mmHg^{-1}$, with a minor variance within each dog but a significant difference amongst the four dogs (p<0.05). No significant change in the ventilatory response to CO_2 was observed after octreotide.

We conclude that intravenous octreotide alters the pattern of breathing but preserves minute ventilation; peripheral administration of octreotide does not influence the ventilatory response to CO_2.
Eur Respir J 1998; 11: 1376–1381.

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Somatostatin is a tetradecapeptide with a wide distribution both through the central nervous system and extraneurally [1]. It exerts potent inhibitory effects in a number of organ systems including basal and secretagogue-stimulated growth hormone (GH) secretion *in vivo* and *in vitro* [1–4]. Somatostatin immunoreactive nerve cell bodies and terminals have been observed in the respiratory nuclei (ventrolateral and ventral subnuclei) of the solitary tract [5]. Intracisternal injection of somatostatin in anaesthetized rats produces an expiratory apnoea that is irreversible [5]. The latency to this apnoea can be shortened by chemoreceptor stimulation with hypoxia and hypercapnia [6]. The somatostatin-induced apnoea can be blocked by naloxone [7]. Interestingly, somatostatin and enkephalin-like immunoreactivity are frequently co-localized in the caudal rat brain-stem [8]. Direct application of somatostatin into the region of the nucleus paragigantocellularis lateralis and nucleus reticularis lateralis [9] produces apnoea. Somatostatin injection in these sites also produces blunting of the ventilatory response to hypoxic and hypercapnic stimuli [9].

Remodelling of the somatostatin molecule has led to the discovery of octreotide (SMS 201-995), a long-acting somatostatin analogue [10]. Octreotide selectively inhibits GH secretion and has a minimal effect on other hormones, such as insulin and glucagon, that are inhibited by somatostatin [11]. This somatostatin analogue is now an important therapeutic agent in the management of acromegaly and some intestinal conditions [12, 13].

There is limited information available on the effect of somatostatin or its analogue in human breathing. One study in normal subjects has reported that intravenous somatostatin attenuates the ventilatory response to hypoxia but not hypercapnia [14]. Long-term administration of octreotide was reported to ameliorate notably the dyspnoea on exertion in a case of cirrhotic hypoxaemia [15]. Another group has observed that somatostatin increases the ventilatory roll-off with sustained hypoxia in humans [16]. Octreotide therapy in patients with acromegaly is associated with a reduction in apnoea frequency [17]. This effect was particularly manifest in the subgroup of patients with central apnoea, a sleep-breathing disorder strongly linked to abnormal central control of breathing [18, 19].

It is possible that peripherally administered somatostatin analogue may influence the control of breathing. In order to test this hypothesis, we examined the effect of intravenous octreotide, at two dose levels, on respiratory pattern and the ventilatory response to hypercapnia in the conscious dog.

Methods

Studies were performed in four unanaesthetized conscious adult dogs (20–30 kg body weight). The dogs had a permanent side-hole tracheal fistula allowing intubation of the trachea for connection to the respiratory measurement apparatus. The dogs were trained to lie quietly on their right side for 2–3 h in the afternoon. The laboratory temperature was kept in the range of 19–21°C.

In order to ensure that all studies were completed in the awake state, electroencephalogram (EEG) signals, with superimposed electro-oculogram (EOG) signals, were obtained *via* a pair of surface electrodes attached to the lateral aspect of the frontal bone.

Respiratory air flow was measured *via* an endotracheal tube with a pneumotachograph attached to a differential pressure transducer (Validyne DP45-14, with Validyne CD103 carrier demodulator; Validyne, Northridge, CA, USA). Tidal volume (V_T) was obtained by electronic integration (Grass 7P10C, West Warwick, RI, USA) of the air flow signal, which was calibrated using a 0.5 L syringe. Tracheal gas was sampled continuously to monitor expired end tidal CO_2 tension (P_{ET,CO_2}) (Capnograph infra-red CO_2 analyser, Hewlett Packard, Andover, MA, USA). During the test of ventilatory response to CO_2, P_{ET,CO_2} was also sampled and recorded when the animal was switched to breathe a bag containing ~7% CO_2 in oxygen (see below).

Signals of P_{ET,CO_2}, EEG, and EOG, respiratory flow and V_T were recorded continuously throughout each experiment on a polygraph recorder (Grass 7D).

In addition, the respiratory flow and P_{ET,CO_2} were digitized at 125 Hz (NEC APC IV computer, Analog Devices RTI-815A analogue-to-digital converter (Norwood, MA, USA). The following were calculated and monitored on the computer screen on a breath-by-breath basis: V_T, ventilation ($\bar{V}'$), respiratory rate (RR) and P_{ET,CO_2}. All values were stored on hard disk in real time throughout each experiment for subsequent analysis.

Experimental protocol

After finalizing the apparatus settings, each dog was observed until they were quietly breathing. A sequence of continuous respiratory recording and computer sampling was than started. This sequence included a 3 min baseline recording followed by a test of ventilatory response to CO_2 (see below). Following the test when the dog's ventilation had returned to baseline (usually in 5–7 min), the same sequence was repeated. A total number of four such experimental sequences were carried out (control period).

The dogs then received a 5 mL injection of either octretide (0.1 mg or 0.5 mg) or normal saline (see below). Ten mins after the injection, the same experimental sequences were performed (test period).

In order to reveal any effect of octreotide on breathing pattern or ventilatory response to CO_2, one of the following was administered intravenously through the left cephalic vein on each experimental day: normal saline only, 0.1 mg octreotide in normal saline and 0.5 mg octreotide
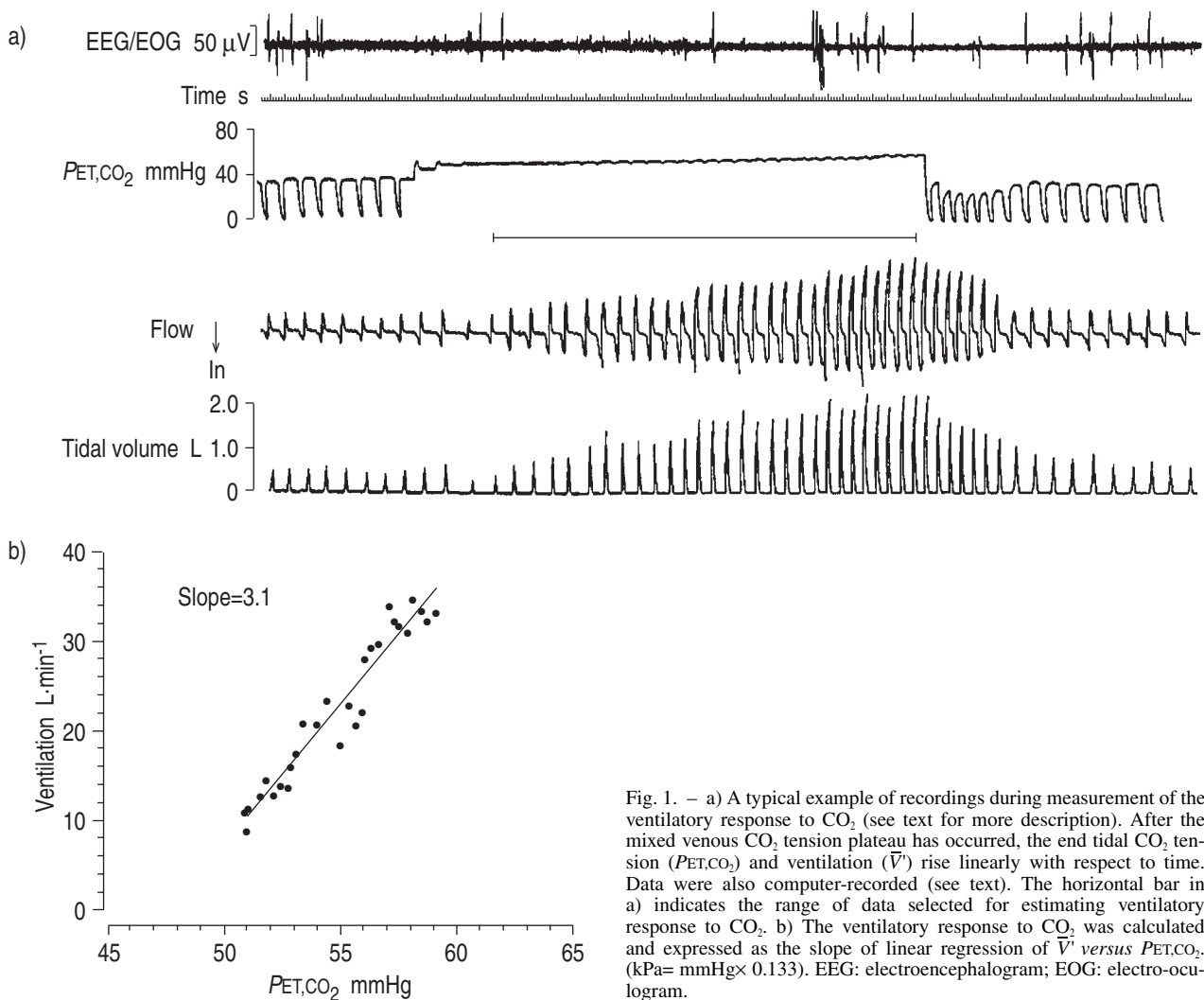

Fig. 1. – a) A typical example of recordings during measurement of the ventilatory response to CO_2 (see text for more description). After the mixed venous CO_2 tension plateau has occurred, the end tidal CO_2 tension (P_{ET,CO_2}) and ventilation ($\bar{V}'$) rise linearly with respect to time. Data were also computer-recorded (see text). The horizontal bar in a) indicates the range of data selected for estimating ventilatory response to CO_2. b) The ventilatory response to CO_2 was calculated and expressed as the slope of linear regression of $\bar{V}'$ *versus* P_{ET,CO_2}. (kPa= mmHg× 0.133). EEG: electroencephalogram; EOG: electro-oculogram.

in normal saline. All injections contained a total volume of 5 mL and were administered over a one minute period.

The experimental protocol was carried out for each dog on three separate days, 1 day for each of the three injections. The drug used on a given day was chosen randomly with the investigator blind to the injection contents.

Ventilatory Response to CO_2. Ventilatory response to CO_2 (progressive hyperoxic hypercapnia) was examined using the Read method [20]. Briefly, when the ventilation and PET,CO_2 were stabilized, the animal was switched from breathing room air to rebreathing from a plastic flexible bag containing ~7% CO_2 in oxygen. A satisfactory mixed venous CO_2 tension (Pv,CO_2) plateau was seen on the chart record two or three breaths after the onset of rebreathing. The animal was then allowed to breathe from the bag quietly for 3–4 min, and then switched back to room air (fig. 1). Because the initial gas mixture contained ~93% oxygen, the dogs remained hyperoxic throughout the rebreathing period.

Data analysis

In order to determine whether any injection results in respiratory pattern changes, we calculated the mean values of VT, RR, $\overline{V}'$ and PET,CO_2 in each of the 3 min periods of breathing sampled during both control and test period. These mean values were then used as the raw data for each dog for the analysis of variance. Therefore, for each breathing parameter, there were 96 observations in total; four dogs with each parameter measured at four time intervals before and after each of three conditions (control, octreotide 0.1 mg, octreotide 0.5 mg). An example of this sampling for one parameter, ventilatory response to CO_2 is shown in table 1.

Table 1. – Individual and group mean data for ventilatory response to CO_2 (L·min^{-1}·mmHg^{-1})

	Dog 1		Dog 2		Dog 3		Dog 4	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Saline	2.6	2.7	2.8	1.8	1.0	0.9	3.2	2.8
	1.6	3.0	2.3	3.6	1.6	1.8	3.4	2.7
	1.7	1.7	2.4	2.4	1.4	1.0	3.1	3.5
	2.0	2.1	2.7	3.4	1.8	1.2	3.1	3.0
Mean	2.0	2.4	2.6	2.8	1.5	1.2	3.2	3.0
CV %	21	24	10	30	22	34	5	12
0.1 mg	2.5	1.7	1.3	1.9	1.0	1.3	2.5	2.5
Octreotide	2.6	2.0	1.3	2.3	1.0	1.3	2.5	2.1
	2.6	2.2	1.2	2.2	1.0	1.1	2.5	2.4
	2.7	1.7	1.7	2.9	1.1	1.3	2.4	2.2
Mean	2.6	1.9	1.4	2.3	1.0	1.2	2.5	2.3
CV %	4	12	18	19	4	10	3	8
0.5 mg	2.4	2.0	2.0	3.5	1.6	1.9	1.8	2.4
Octreotide	2.5	2.6	2.2	3.2	2.0	2.1	2.7	2.2
	2.7	2.4	3.2	3.0	3.7*	2.5	1.8	3.3
	2.6	2.0	2.8	2.3	1.6	2.1	2.0	2.6
Mean	2.6	2.3	2.6	3.0	1.7	2.1	2.1	2.6
CV %	5	12	22	16	12	12	21	17

*: outlier was excluded from calculation. CV: coefficient of variation (=SD/mean ×100%); Pre and Post: pre- and post-injection.

To evaluate any changes in ventilatory response to CO_2 induced by octreotide, linear regression analyses of $\overline{V}'$ against PET,CO_2 (fig. 1) were performed for all CO_2 test runs before and after injection of octreotide or saline. The slope values of these regressions were compared by a two-way analysis of variance. Statistical significance was considered at $p < 0.05$.

Results

All dogs tolerated the experimental protocol with no observed behaviour disturbance during injection or any other procedure. A total number of 96 respiratory measurements and 96 ventilatory response to CO_2 records were obtained and analysed.

Breathing pattern changes

Placebo. As expected, injection of saline had no effect on minute ventilation (fig. 2c, $p > 0.05$). However, saline injection led to an increase (11%) in RR and a concomitant fall (9%) in VT. Although the shift of RR-VT relationship induced by saline injection reached a significant level in our experiment (fig. 2a and b, $p < 0.05$), the dogs tolerated the injection well, and no behavioural disturbances were observed.

Octreotide. Although both *i.v.* doses (0.1 mg and 0.5 mg) of octreotide did not significantly alter the minute ventilation in the conscious dog (fig. 2c, $p > 0.05$), octreotide (0.5 mg) had an effect on the RR-VT relationship, opposite to that seen with saline injection. Octreotide injection (0.5 mg) led to a 23±6% reduction of RR and a 16±6% concomitant increase in VT. The decrease in RR and increase in VT as a result of the octreotide injection became more pronounced when the injection dose increased from 0, 0.1 to 0.5 mg (fig. 2a and b, $p < 0.05$).

Ventilatory responses to CO_2

Normal values. The values of ventilatory responses to progressive hyperoxic hypercapnia under normal conditions (control) varied widely (from 1.0 to 3.4 L·min^{-1}·mmHg^{-1} 2.1±0.67 (mean±SD), 47 measurements in four dogs, one outlier excluded). However, the ventilatory response to CO_2 was relatively stable in individual dogs for a given experimental day (with an average coefficient of variation of 12±2.3%). Significantly different response values were seen among the dogs (table 1, $p < 0.05$).

Effects of saline and octreotide injection. Injection of saline did not affect the ventilatory response to progressive hyperoxic hypercapnia (2.29±0.18 *versus* 2.35±0.22 (SEM) L·min^{-1}·mmHg^{-1}, $p > 0.05$). Also, the octreotide injection at either dose did not significantly alter the ventilatory response to CO_2 (1.86±0.18 *versus* 1.93±0.13 for 0.1 mg octreotide; 2.27±0.13 *versus* 2.5±0.12 for 0.5 mg octreotide; $p > 0.05$; fig. 3).

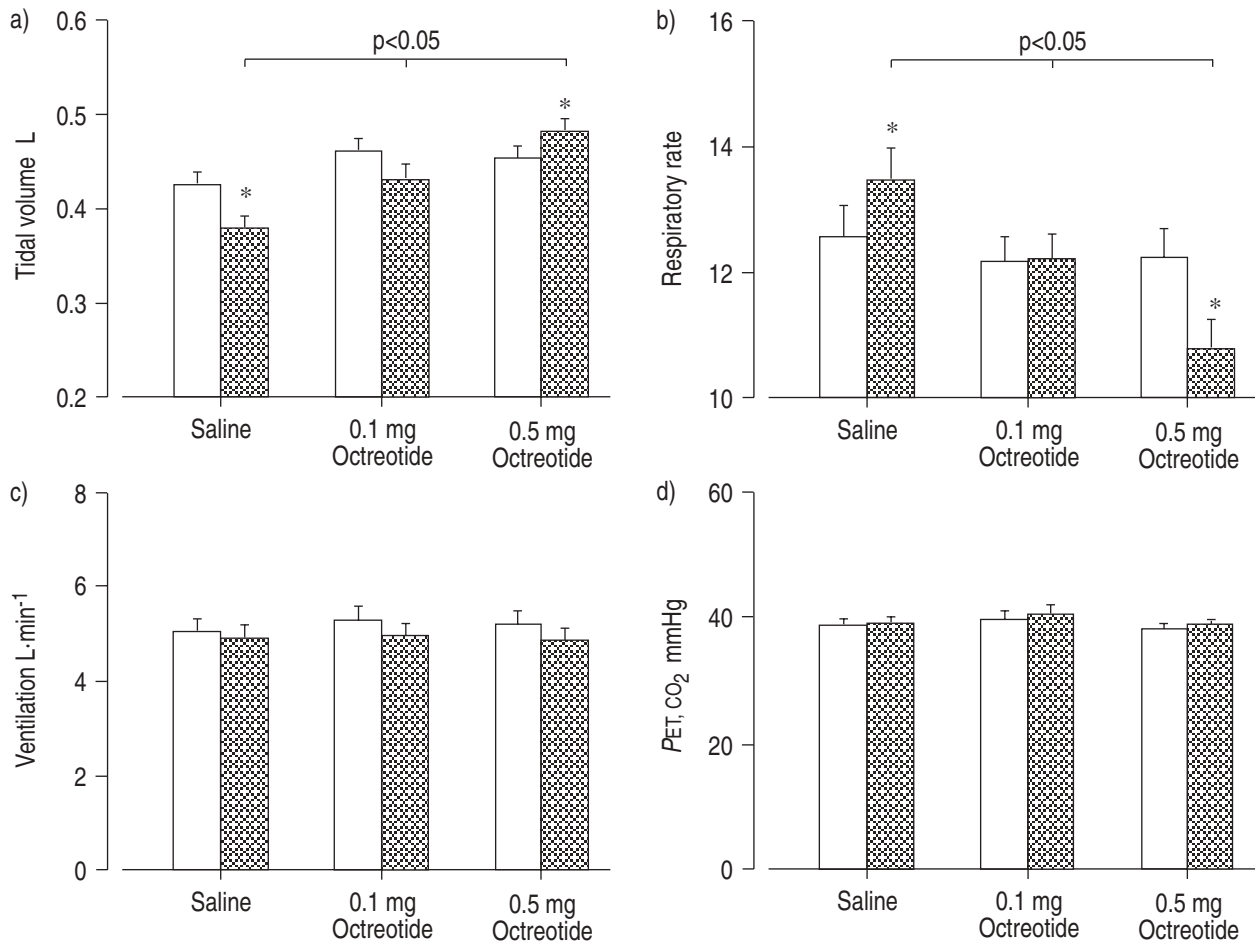The PET,CO_2 was not significantly changed under any injection conditions ($p > 0.05$, fig. 2d).

Fig. 2. – The effects of saline, 0.1 mg and 0.5 mg octreotide injections on a) tidal volume, b) respiratory rate, c) ventilation and d) expired end tidal CO_2 tension (PET,CO_2). ☐ mean values (with SEM) of variables before injection; ▨ mean value (with SEM) of variables after injection. Note that, in contrast to saline injection, octreotide injection (0.5 mg) led to a significant decrease in respiratory rate and a concomitant rise in tidal volume, resulting in no significant change in ventilation. *: significant change *versus* pre-injection level ($p<0.05$). (kPa=mmHg×0.133).
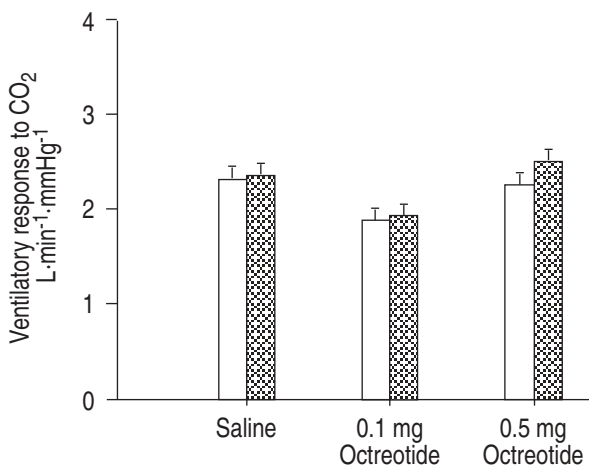

Fig. 3. – The effects of saline, 0.1 mg and 0.5 mg octreotide injections on ventilatory response to CO_2. ☐ : group mean slope (with SEM) of pre-injections. ▨ : group mean slope (with SEM) of post-injections.

Discussion

These experiments have demonstrated that the intravenous administration of the somatostatin analogue octre-

otide alters the breathing pattern in conscious dogs. Saline injection produced an increase in respiratory rate and decrease in tidal volume, presumably due to a subtle behavioural response in these animals. In contrast, octreotide 0.5 mg produced a reduction in RR which was balanced by an increase in VT, resulting in no change in minute ventilation. However there was no consistent effect on the ventilatory response to carbon dioxide following octreotide.

The limited effects of intravenous octreotide on breathing in these dogs are in contrast to the marked effects on respiration described with the intracisternal administration of somatostatin in rats. FUXE *et al.* [21] first reported that somatostatin produced irreversible apnoea that was attriuted to a depression of respiratory centres. In these animals, apnoea was preceded by slow deep breathing, a similar pattern (reduced RR, increased VT) as observed in our study. These findings were later confirmed by KALIA *et al.* [5] who applied somatostatin into the ventricular system in alpha-chloralose anaesthetized rats. The apnoea induced by somatostatin could not be prevented by bilateral cervical vagotomy or decerebration below the level of the inferior colliculus. In view of the existence of somatostatin immunoreactive neurones and terminals in the nucleus tractus solitarius (NTS), these authors proposed

that the site of action of somatostatin was in the dorsal respiratory neuron group of the medulla oblongata. However, YAMAMOTO *et al.* [22] subsequently reported that apnoea could only be induced by micro-injection of somatostatin into the region of nucleus paragigantocellularis lateralis (nPGL) in anaesthetized cats. No inhibitory effects of somatostatin on respiration were seen after micro-injection into the NTS [23]. In humans, a 40 min infusion of somatostatin (1 mg·h⁻¹) produced a small reduction in resting ventilation but did not affect the ventilatory response to CO_2 [14]. Other workers observed a trend towards decreased minute ventilation 10 min after a bolus of 0.25 mg and 0.7 mg·h⁻¹ infusion of somatostatin [16]. However, it is important to recognize that all these animal studies were performed under anaesthetized conditions, and the drug used in both animal and human studies was somatostatin rather than its analogue, octreotide, which may have different physiopharmacological effects. Moreover, it is difficult to compare effects of drugs administered by different routes, such as intracisternal *versus* intravenous injection.

The change in respiratory pattern that we observed following octreotide injection is consistent with an inhibitory effect on RR in these conscious animals and not due to the injection procedure itself. The current experiment does not provide a mechanism or site for this effect. Octreotide could potentially influence breathing by either a peripheral or a central mechanism. Evidence supporting a central mechanism is twofold. Firstly, results from studies of intracisternal administration of somatostatin [5, 21] are consistent with the relatively minor effects observed in this study. Secondly, clinical use of octreotide as a growth hormone lowering agent leads to a reduction of apnoea severity in acromegaly, particularly in patients with central apnoea [17]. One possible mechanism for this reduction in apnoea severity is that octreotide may centrally reduce chemoreceptor gain, which is increased in patients with central apnoea [17, 19]. In addition, intraperitoneal injections of octreotide alter sleep architecture in rats [24], also supporting the central effects of peripherally administered octreotide.

However, in our study, the lack of a ventilatory response to CO_2 is in contradiction with a central effect on breathing. Alternatively, octreotide may be a somatostatin analogue with only limited effects on somatostatinergic respiratory neurones. Recently, several subtypes of somatostatin receptor have been identified with potentially different effects within the body [25]. For example, administration of CTOP, a somatostatin analogue with μ-opioid receptor antagonist activity and minimal action on somatostatin receptors, leads to an increase in RR in swine [26], opposite to our findings with octreotide in dogs. Also, it is unknown whether sufficient amounts of octreotide cross the blood-brain barrier to affect central control of breathing. It is certainly conceivable that the observed effect of octreotide may be peripheral as there is a wide distribution of somatostatin receptors in peripheral tissue.

The present study demonstrates no evidence of octreotide affecting the ventilatory response to CO_2 (fig. 3). Although three out of four dogs showed a slight increase in the ventilatory response to CO_2 following 0.5 mg octreotide injection (table 1), these increases in response to CO_2 were not significant. One possible explanation for

the lack of effect of octreotide on central chemoreceptors might be the dog's conscious status. CHEN *et al.* [9] suggested that the conscious status can markedly alter the effect of somatostatin on respiration. They successfully demonstrated that 0.6 nmol of somatostatin injected into the areas of the nucleus reticularis lateralis (nRLt) in the rat during intermediate or deep anaesthesia resulted in apnoea within a short period of time. The same result was also seen in unanaesthetized decerebrated rats. However, repeated application of 0.6–1.2 nmol of somatostatin during conscious conditions did not induce any significant effects on respiration. Species differences may also contribute to the differing observations reported between human and dogs. The dogs in our study did not have acromegaly, and the interaction of octreotide and respiratory control may be different in humans or other animals with high growth hormone levels.

We also observed a considerable variability in the ventilatory response to carbon dioxide between dogs (table 1), similar to observations in humans [27]. The ventilatory response to CO_2 has a wide normal range in humans, with a variability secondary to a number of factors including age and familial background [27, 28]. In our study, age was unlikely to account for the variability as the oldest dog (12 yrs old) had the highest ventilatory response (dog 4 in table 1).

In conclusion, our findings show that, whereas octreotide had no consistent effect on ventilatory response to carbon dioxide, it produced an alteration in breathing pattern with reduced respiratory rate and increased tidal volume in conscious dogs. Further studies are required to investigate the mechanism for these changes in breathing.

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