Effect of aminophylline and verapamil upon diaphragmatic force generation in dogs

E.L. De Vito, A.J. Roncoroni


ABSTRACT: The effects on the diaphragm of verapamil (VPM) and aminophylline (AMP) were studied in dogs during stimulation of phrenic nerves at the 5th cervical roots (5th-PS) or transvenously at the trunk (T-PS). Transdiaphragmatic pressure (Pdi)/frequency curves were constructed. Our findings were: 1) AMP increased Pdi at all stimulation frequencies (p<0.01) during 5th-PS but only at 10–30 Hz during T-PS; 2) in other dogs infusion of VPM (0.14 mg·kg⁻¹·min⁻¹) decreased Pdi at all frequencies (p<0.025) without change in diaphragmatic blood flow; 3) the effects of VPM were completely reverted by AMP; 4) after a larger amount of AMP, infusion of VPM (0.21 mg·kg⁻¹·min⁻¹) decreased Pdi at all frequencies. Since these drugs have several mechanisms of action and do not show mutual blocking effect, different action sites are suggested.


Aminophylline (AMP) is one of the few drugs used for treatment of chronic obstructive pulmonary disease (COPD). In the past, a stimulatory effect upon the central respiratory controllers and bronchodilation were the favoured mechanisms of its action. Since the establishment of its beneficial effects upon force developed by the fresh or fatigued diaphragm it use has increased, as inspiratory muscle fatigue may compromise further ventilatory incapacity in COPD. The mechanism of this effect remains, however, unclear. In isolated rat hemidiaphragm Varagic and Kentera [1] found that AMP increases twitch tension and maximal velocity of tension increase (dp/dt) max, an effect markedly dependent upon Ca²⁺ concentration in the bath. This effect was not observed when Ca²⁺ was absent or verapamil (VPM) was added. Similarly, Aubin et al. [2] showed that AMP infusion in dogs increased tension developed by fresh diaphragms stimulated in situ at 20 Hz. This effect was not obtained under previous VPM infusion. Other investigators [3] also working with diaphragms of dogs in situ, stimulated through the nerves at 1–40 Hz, did not find that the beneficial effects of AMP upon muscle contractility were affected by VPM infusion. Therefore, they concluded that AMP action upon the diaphragm does not require normal operation of calcium channels. Recent evidence [4, 5] suggests that AMP stimulates the Na-K pump.

Our study was conducted to investigate the effects and interaction of AMP and VPM upon diaphragmatic response to phrenic stimulation by single twitch and tetanic pulses at several frequencies. The drugs were used singly and combined at two different dosages. Since both are vasoactive drugs diaphragmatic blood flow (Qdi) was measured to evaluate possible changes. The study was intended to clarify the previously quoted [2, 3] converse results also obtained, in dog diaphragm in situ, with similar methods.

Materials and methods

Thirty dogs from 7.5–30 kg body weight (BW) were anaesthetized with sodium pentobarbital (30 mg·kg⁻¹) i.v., intubated and mechanically ventilated during the surgical procedure. Anaesthesia was maintained with constant pentobarbital venous infusion with a Harvard pump. The femoral artery was catheterized for blood pressure monitoring and sampling. Rectal temperature was monitored and maintained constant.

Oesophageal and gastric pressures were measured by latex catheters and balloons (5 cm long) placed in the middle third of the oesophagus and in the stomach. Each catheter was connected to one side of a differential pressure manometer (Validyne MP-45) to obtain transdiaphragmatic pressure (Pdi). They were also connected to two similar differential pressure transducers recording transpulmonary pressure (Ptp, oesophageal vs tracheal) and gastric pressure (Pga) against atmospheric.

Phrenic nerve stimulation was carried out: a) in 18 dogs at the 5th cervical roots (5th-PS); and b) in 12 dogs...
at the intrathoracic nerve trunks (T-PS). Both phrenic nerves at the fifth cervical root (5th-PS) were isolated and platinum electrodes under oil were placed and protected with gauze pads. Fish hooks were placed, through superior laparotomy, in the anterior muscle part of both diaphragms to record muscle potential (EMG). The surgical incision was carefully closed and residual air aspirated.

The nerves were stimulated by 0.1 ms pulses during about 2 s at frequencies of 10, 30, 60, 100 Hz while Pdi was being measured. After the voltage provoking maximal Pdi at 100 Hz was determined, current intensity was raised 10–20% (usually to 10–12.5 volts) to ensure supramaximal stimulation of the muscle innervated by the 5th cervical roots. Single twitch Pdi were also measured with supramaximal stimuli of 0.1 ms duration. Peak twitch tension (PTT), maximal velocity of tension increase (dp/dt max) and relaxation speed (MxRx) were measured. Since changes in EMG were minimal during tetanus it was assumed that stimulating electrode position was maintained. The study was conducted during spontaneous breathing and positive cornual reflex.

It is well known that the Pdi developed depends on the initial diaphragmatic length. This is influenced by changes in the lung volume and abdominal pressure under the effect of the abdominal muscle’s state of contraction. Electrical stimulation was applied at functional residual capacity (FRC) with the airway occluded. The constancy of FRC was evaluated by Ptp level at end expiration and abdominal muscle contraction by monitoring Pga. Both Ptp and Pga were stable before stimulation. Since only the 5th cervical roots were stimulated, diaphragmatic shortening was limited to anterior parts so that contraction was submaximal, in relation to the whole muscle. The consistency of diaphragmatic activation (Edi) ensured that the responses after drug administration were comparable.

In 12 dogs, bilateral T-PS was obtained by introducing 2 catheters into the internal left jugular vein and advancing them to the point of maximal Pdi response at 100 Hz [6]. To register spontaneous electrical activity of the anterior rectus abdominis fish hooks were placed through a small incision. A cast was placed covering the lower thorax and abdomen.

In 6 other dogs, the jugular vein was catheterized and a Swan-Ganz catheter placed in the pulmonary artery trunk. A Cordis shepherd hook catheter was advanced, through the femoral vein under fluoroscopy, into the left diaphragmatic vein. Cardiac output (CO) was measured by thermodilution. Left hemidiaphragmatic blood flow was measured by blood collection in a calibrated vessel. In order to compensate for catheter resistance internal diameter (ID): 1.2 mm) a negative pressure was produced placing the catheter tip 45 cm below the horizontal plane via the dog’s back. The means of three measurements, with a variation coefficient less than 10%, were used for calculation of the two variables. Diaphragm blood flow (Qdi) was reproducible. Specific vascular diaphragmatic conductance (VCDi) was estimated by the following equation:

\[
\text{Qdi} \text{ ml}\cdot\text{min}^{-1}\cdot\text{g}^{-1}/\text{mean arterial pressure (MAP) mmHg}
\]

At the end of the experiment the venous catheter position was confirmed by fluoroscopy and India ink was injected through it. At post mortem examination it was observed that the whole left hemidiaphragm was coloured. The muscle was carefully dissected and weighed with and without the central tendon in order to calculate Qdi per gram of muscle. The result obtained, 4.91±0.16% BW, is similar to published results [7].

Protocol 1

In 12 dogs, 6 (5th-PS) and 6 (T-PS), AMP was injected intravenously in a bolus dose of 15 mg•kg⁻¹, after basal stimulated Pdi determinations. Twenty minutes later Pdi/Frequency curves were obtained.

Protocol 2

In 18 dogs, a continuous venous infusion of VPM (0.14 mg•kg⁻¹•min⁻¹) was started after basal measurements of Pdi. When mean arterial pressure (MAP) and heart rate (HR) were about half of basal values during 15 min, Pdi measurements were repeated. Immediately afterwards, AMP was given in a bolus injection of 15 mg•kg⁻¹. After 20 min samples for AMP blood levels were obtained and new Pdi measurements carried out while VPM infusion was continued. When VPM infusion was stopped HR recovered basal values but MAP remained low. In 12 dogs two hours later, AMP bolus injection was repeated in the same amount (AMP₂) and new Pdi values (5th-PS) and AMP plasma levels were obtained 20 min later. Infusion of VPM was reinstalled, now at 0.21 mg•kg⁻¹•min⁻¹, and after a fall of HR new Pdi measurements were carried out.

Protocol 3

In 6 dogs, CO and Qdi were measured before and after VPM and AMP infusions, reproducing the procedure described in Protocol 2 with only the first dosage schedule.

The animals were studied in supine position breathing air, with normal arterial oxygen tension (PaO₂) and acid-base levels. Each Pdi is the mean of the three measurements. All variables were recorded in Physiograph MK-IV P and abdominal muscle EMG also amplified on audio-system.

Results are shown as mean±standard error of mean (SEM). Statistical analysis was performed by Student’s test for paired data or analysis of variance when appropriate.

Results

With 5th-PS, basal Pdi at 100 Hz was 26.8±1.5 cmH₂O while with T-PS the corresponding value was 73.8±2.9 cmH₂O, similar to published results [8, 9].
Pdi values expressed as % basal Pdi at 100 Hz. Results are shown as mean±SEM. NS: not significant; AMP: aminophylline; 5th-PS: stimulation of phrenic nerves at 5th cervical roots; T-PS: stimulation of phrenic nerve at the trunk.

Table 2. – Effects of VPM₁ and AMP₁ upon Pdi/frequency relations

<table>
<thead>
<tr>
<th>Hz</th>
<th>VPM₁ Basal</th>
<th>VPM₁ AMP₁ Basal</th>
<th>VPM₁ AMP₁ Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>22.2±2.0</td>
<td>18.0±1.8</td>
<td>22.2±2.5</td>
</tr>
<tr>
<td>30</td>
<td>86.4±2.1</td>
<td>76.6±3.7</td>
<td>81.6±3.8</td>
</tr>
<tr>
<td>60</td>
<td>94.5±1.0</td>
<td>75.4±2.3</td>
<td>88.3±3.7</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>79.2±3.0</td>
<td>94.4±3.9</td>
</tr>
<tr>
<td>10/60</td>
<td>0.24±0.02</td>
<td>0.24±0.02</td>
<td>0.25±0.02</td>
</tr>
</tbody>
</table>

5th-PS: Basal vs VPM₁, p<0.0005; VPM₁ vs VPM₁+AMP₁, p<0.025; Basal vs VPM₁+AMP₁, NS; 10/60 Hz NS. T-PS: Basal vs VPM₁, p<0.0025; VPM₁ vs VPM₁+AMP₁, p<0.0025; Basal vs VPM₁+AMP₁, NS; 10/60 Hz NS. Pdi values expressed as % basal Pdi at 100 Hz. Results are shown as mean±SEM. NS: not significant; VPM: verapamil. For other abbreviations see legend to table 1.

Table 3. – Effect of VPM upon Pdi/frequency relation of AMP pretreated dogs

<table>
<thead>
<tr>
<th>Hz</th>
<th>AMP₂ Basal</th>
<th>AMP₂ AMP₁ Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>28.7±2.1</td>
<td>24.3±2.4</td>
</tr>
<tr>
<td>30</td>
<td>89.1±1.3</td>
<td>76.4±4.4</td>
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<tr>
<td>60</td>
<td>97.7±1.5</td>
<td>83.6±4.8</td>
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<tr>
<td>100</td>
<td>100</td>
<td>83.9±4.1</td>
</tr>
<tr>
<td>10/60</td>
<td>0.29±0.02</td>
<td>0.29±0.02</td>
</tr>
</tbody>
</table>

Pdi value expressed as % basal Pdi at 100 Hz. Results are shown as mean±SEM. For abbreviations see legends to tables 1 and 2.

Protocol 2

Infusion of VPM₁ (5th-PS) induced a markedly significant fall in MAP from 135±4.3 to 77.5±3.8 mmHg (p<0.0005) unchanged by AMP₁ or AMP₂. After VPM₁ infusion, MAP decreased to 55.5±5.0 mmHg (p<0.0005). Basal HR was 179±11.6 beats·min⁻¹ decreased to 102±7.2 beats·min⁻¹ (p<0.0005) during VPM₁ and remained unchanged after AMP₁ injection. With higher AMP concentration HR was 167±11.9 beats·min⁻¹ (similar to basal) and fell to 119±9.1 beats·min⁻¹ (p<0.0005) during VPM₁ infusion. Similar results were found during T-PS.

Infusion of VPM₁ (5th-PS) decreased Pdi at all stimulation frequencies (p<0.0005) while AMP₁ injection (plasma level: 19.4±4.2 mg·l⁻¹) completely reverted this effect (p<0.0025) so that Pdi/frequency curves were no different from basal (fig. 1). Neither VPM₁ nor AMP₁ changed the 10/60 Hz relation (table 2). Results after T-PS were similar (table 2). After injection of AMP₂ (plasma level: 35.4±6.8 mg·l⁻¹) VPM₁ infusion decreased Pdi at all frequencies (p<0.05) (fig. 2). The Pdi relations at 10/60 Hz were not modified by AMP₁ or VPM₁. Peak twitch tension and speed of tension development were decreased by VPM₁, to 85.3±5.7% (p<0.05) and 84.6±5.5% (p<0.01) of basal while relaxation was not modified. After AMP₂, these changes were completely reverted so that basal values were recovered (table 4).

With T-PS, peak twitch tension (PTT), dp/dt max and
AMINOPHYLLINE, VERAPAMIL AND DIAPHRAGMATIC FORCE GENERATION

459

n=12

Fig. 1. - Effects of VPM and AMP upon Pdi/frequency curves (5th-PS). Plasma level of AMP; 19.4±4.2 mg·l⁻¹, VPM, infusion rate: 0.14 mg·kg⁻¹·min⁻¹.

n=12

Fig. 2. - Effects of VPM and AMP upon Pdi/frequency curves (5th-PS). Plasma level of AMP; 35.4±6.8 mg·l⁻¹, VPM, infusion rate: 0.21 mg·kg⁻¹·min⁻¹.

Table 4. - Effects of VPM upon Pdi single twitch

<table>
<thead>
<tr>
<th>5th-PS</th>
<th>T-PS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>VPM</td>
</tr>
<tr>
<td>PTT</td>
<td>85.3±5.7</td>
</tr>
<tr>
<td>dp/dt max</td>
<td>84.6±3.5</td>
</tr>
<tr>
<td>MxRx</td>
<td>82.8±9.4</td>
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</tbody>
</table>

PTT: peak twitch tension; dp/dt max: maximal speed of Pdi generation; MxRx: maximal relaxation speed. Values are expressed as % of basal. Results are as mean±SEM. For other abbreviations see legends to tables 1 and 2.

Table 5. - Haemodynamic data (Protocol 3)

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>VPM</th>
<th>VPM+AMP</th>
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</thead>
<tbody>
<tr>
<td>CO ml·kg⁻¹·min⁻¹</td>
<td>146±27</td>
<td>150±52</td>
<td>130±25</td>
</tr>
<tr>
<td>MAP mmHg</td>
<td>151.5±15.4</td>
<td>87.4±10.6</td>
<td>72.1±6.7</td>
</tr>
<tr>
<td>HR beats·min⁻¹</td>
<td>177.2±21.7</td>
<td>83.3±11.0</td>
<td>63.2±8.6</td>
</tr>
<tr>
<td>Qdi ml·min⁻¹·g⁻¹</td>
<td>0.12±0.01</td>
<td>0.13±0.02</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td>VCDi ml·min⁻¹·g⁻¹·mmHg⁻¹</td>
<td>8.5±1.6</td>
<td>15.9±2.9</td>
<td>21.7±4.5</td>
</tr>
</tbody>
</table>

Results are shown as mean±SEM. CO: cardiac output; MAP: mean arterial pressure; HR: heart rate; Qdi: diaphragm blood flow; VCDi: vascular/diaphragmatic conductance. For other abbreviations see legends to tables 1 and 2. Statistical analysis. MAP: B vs VPM, p<0.0025; HR: B vs VPM, p<0.0025; B vs VPM+AMP, p<0.05. VCDi: B vs VPM, p<0.0125; VPM vs VPM+AMP, p<0.025. All others ns.

MxRx were decreased by VPM₁ (p<0.025) while AMPprovoked an increase over basal values (table 4).

Protocol 3

Basal CO (variance analysis: F=0.08) did not change during VPM infusion or after AMP (table 5). Mean arterial blood pressure showed similar changes to those observed in Protocol 2, i.e. the decrease induced by VPM was not corrected by AMP. Mean basal specific Qdi, 0.12±0.01 ml·min⁻¹·g⁻¹ coincided with results obtained collecting venous outflow from diaphragmatic muscle strips [10]. Control Qdi was not significantly modified by VPM or AMP (variance analysis: F=0.46) (table 5). Mean control specific VCDi was 8.5±1.6 ml·min⁻¹·g⁻¹·mmHg⁻¹·10⁴. Basal VCDi was increased by VPM (p<0.0125) and further more by AMP (p<0.025). Pressure/VCDi curve showed a slope of -0.14±0.04 ml·min⁻¹·mmHg⁻¹ (r=0.69, p<0.01).
Discussion

Evaluation of the method

Force developed by the diaphragm under tetanus or single twitch stimulus was estimated by Pdi determinations. Before accepting modification in diaphragmatic contractility (DC) as the cause of Pdi alterations, possible changes in lung volume or chest-abdomen configuration should be considered, otherwise different muscle fibre length may well be the reason for Pdi changes. Electrical stimulation at the 5th phrenic roots was carried out at end-expiratory level (FRC) with constant recording of transpulmonary pressure (Ptp) which did not change more than 2 cmH₂O during the experiment. Expiratory gastric pressure (Pga) just before stimulation did not change more than 2 cmH₂O either. We believe that abdominal muscle contraction, if present, was probably insufficient to modify diaphragm length. Partial contraction of the muscle may result from this technique, presumably inducing lengthening of unstimulated portions. In order to clarify some of these problems we performed T-PS, restricted lower thoracic and abdominal expansion with a cast and recorded EMG of the rectus abdominis. In these conditions Pdi at 100 Hz was much higher and similar to published values [9] reflecting complete diaphragmatic stimulation. Since Pga and Pdi at end-expiration were unchanged and the EMG of rectus abdominis muscle was silent during expiration, we excluded significant diaphragm lengthening after AMP. Relaxing effects of AMP on the diaphragm have not, to our knowledge, been described. Conversely increase in resting tension was found above 60 mg·l⁻¹ concentration in isolated fibre preparations [11].

Shortening, which decreases Pdi generation, is reduced but not suppressed by the cast [12]. For that reason the Pdi obtained are the result of the contractile properties of the muscle and the force-length characteristics.

Aminophylline effects

After AMP with 5th-PS, Pdi increased at all frequencies while with T-PS the increase was limited to 10–30 Hz (Protocol 1). We believe this difference may be attributed to submaximal stimulation with the 5th-PS technique [13]. With the latter procedure the increase at 10 Hz was 31.6% and with T-PS, 29.8% (table 1). Increase in force generation (FG) after AMP has been reported in similar experimental models. At 24 mg·l⁻¹ AMP plasma level single twitch increased 21% and at 20 Hz, 29% [3]; at 20–30 mg·l⁻¹ Pdi at 10 Hz increased 31.3% [8].

There is general agreement that action of AMP upon contractility is due to a direct action on muscle [11, 14]; the mechanism is, however, unclear. Several in vitro studies were addressed to clarify this point. In rat diaphragm, AMP action was dependent on optimal Ca⁺⁺ concentration and functionally operative Ca⁺⁺ channels since VPM (4.5 mol·l⁻¹) blocked its effects. It was thought that Ca⁺⁺ channel influx was facilitated by AMP. This effect, however, was obtained at AMP concentrations (0.2–2 mmol·l⁻¹=36–180 mg·l⁻¹) much higher than therapeutic levels [1]. Recently, in isolated diaphragmatic fibres, an increase in peak twitch tension was found [11] at therapeutic concentration levels (15 mg·l⁻¹). Since provoked hypocalcaemia decreases Pdi/frequency curves [15] and VPM prevents AMP enhancement of contractility [2] it was accepted that AMP effects are dependent on Ca⁺⁺ influx.

In isolated hamster diaphragmatic strips, AMP at 100 mg·l⁻¹ increased tension and resting membrane potential. This hyperpolarization was attributed to increased K⁺ influx and Na⁺ extrusion [4, 5]. It is possible that the latter effect is Ca⁺⁺ dependent.

Verapamil effects

Decrease in FG during VPM infusion (0.14 mg·kg⁻¹·min⁻¹) is in agreement with peak twitch tension and dp/dt max decrease observed in isolated rat diaphragm [1]. A slight decrease in Pdi at 20 Hz with VPM infusion (0.1 mg·kg⁻¹·min⁻¹) was reported by Aubier et al. [2]. Since the animals had received AMP previously and VPM infusion rate was lower than ours, it is possible to explain the more marked effect obtained in our dogs. Since we induced a marked decrease in arterial pressure and heart rate the possible lowering effect on cardiac output should be ascertained. Skeletal muscle force depends on adequate energy provision and muscle blood flow is related to cardiac output [7]. Neither this last variable nor Qdi were changed by VPM or AMP. On the other hand, a marked decrease in systemic arterial resistance and increase in VCDi was provoked by VPM, an effect enhanced by AMP. The slope of the inverse correlation between MAP and VCDi coincides with that obtained with an electromagnetic flow probe in the phrenic artery [16] and suggests the existence of vascular autoregulation. Changes in FG dependent on Qdi were ruled out (table 6) and, anyway, they would influence endurance rather than response to short isolated tetanus.

Recently, VPM in isolated diaphragm of hamsters was shown to decrease tension and resting membrane potential [4]. Slow inward Ca⁺⁺ currents of sarcolemma are inhibited by VPM, without affecting sarcoplasmatic reticulum Ca⁺⁺ channels in the heart [17]. Both calcium influx and efflux are decreased. Extracellular Ca⁺⁺ levels have a competitive effect on this action and activation of unblocked channels does not seem to be affected [18].

Effects of VPM, however, are not limited to blocking of calcium channels. This drug induces a local anesthetic-like action in frog sartorius muscle, decrease in Na⁺ and K⁺ conductance and membrane excitability [19]. In isolated rat soleus muscle, VPM (0.1 mmol) gradually reduces action potential amplitude during repetitive stimulation, more evidently at high frequencies [20]. In isolated hamster diaphragm VPM decreased resting membrane potential [4]. Fall in Pdi at all stimulation frequencies with preservation of the 10/60 Hz relation, which we found during VPM infusion, suggests global depression in contractility. This is perhaps the result of
local anaesthesia, instead of a selective action upon slow Ca** channels, in which case only low frequency fatigue should be expected. The depressing effect upon twitch tension and speed of force development which we found, cannot be explained by slow Ca** channels inactivation, considering the shorter contraction time [21]. These effects may depend on depression of Na* and K* currents of the muscle cellular membrane [20].

Combined effects of AMP and VPM

In our dogs AMP completely reversed VPM effects upon FG at low and high stimulation frequencies and single twitch. These results differ from those of Varagic and Kentera [1] with single twitch and Aubier et al. [2] during tetanic stimulation only at 20 Hz. On the other hand, in dogs pretreated with AMP at high plasma levels, it was also necessary for a higher AMP infusion rate in order to decrease FG. These results are opposed to those of Di Marco et al. [3] but they used a much lower VPM infusion rate (0.02 mg·kg·min”). In isolated hamster diaphragm, when both VPM and AMP were present in the bath, no changes were found in resting membrane potential and maximal tetanic tension at 100 Hz [4].

The action site of the drugs we used is incompletely known and action mechanisms cannot be explored by were present in the bath, no changes were found in resting membrane potential and maximal tetanic tension at 100 Hz [4].

The action site of the drugs we used is incompletely known and action mechanisms cannot be explored by studies of this type. Effects of VPM upon muscle may be attributed to calcium channel blocking or anaesthetic-like action. On the other hand, AMP effects apparently depend on increased calcium inflow or calcium-dependent increased K* inflow provoking hyperpolarization. This last effect has only been observed in vitro at very high supratherapeutic levels for humans [4]. Our results could be explained by adequate combination of the previous three possible mechanisms.

Acknowledgments: The writers wish to thank M. Rodriguez and A. Brenta for technical assistance and N. Montoya for preparation of typescript.

References


le chiens au cours de la stimulation des nerfs phréniques au niveau des 5èmes racines cervicales (5th-PS) ou par voie transveineuse au niveau du tronc (T-PS). L'on a construit des courbes pression transdiaphragmatique (Pdi) sur fréquence. Nos observations sont les suivantes: 1) l'aminophylline augmente Pdi à toutes les fréquences de stimulation (p<0.01) si l'on stimule 5th-PS mais uniquement à la fréquence de 10 à 30 Hz pour la stimulation T-PS; 2; chez d'autres chiens, l'infusion de VPM (0.14 mg·kg⁻¹·min⁻¹) diminue Pdi à toutes les fréquences (p<0.025) sans modifier le débit sanguin diaphragmatique; 3) Les effets de VPM sont complètement annihilés par AMP; 4) après administration d'une quantité plus élevée d'AMP, l'injection de VPM (0.21 mg·kg⁻¹·min⁻¹) diminue Pdi à toutes les fréquences. Le fait que ces médicaments aient plusieurs mécanismes d'action et n'entraînent aucun effet bloquant mutuel suggère des sites d'action différents.