The effects of the anti-curare agent 4-aminopyridine on the cardiovascular systems of cats and greyhounds under barbiturate-chloralose anaesthesia have been studied. In both species, 4-aminopyridine produced a transient atropine-sensitive decrease in arterial pressure followed by a prolonged adrenergically-mediated increase. In the cat, the cardiac responses to vagal stimulation and the nictitating membrane responses to sympathetic stimulation were augmented after injection of 4-aminopyridine, and the evidence indicated that these effects were the results of increased release of neurotransmitters. In the greyhound, 4-aminopyridine produced increases in left ventricular systolic pressure and dP/dt max, right atrial pressure, stroke volume, myocardial blood flow, myocardial oxygen consumption, external cardiac work, arterial oxygen content and blood haemoglobin. These effects were attributable to facilitation of sympathetic transmission to the blood vessels, heart and spleen. Heart rate was not much affected because facilitation of vagal transmission to the S-A node counteracted the increased sympathetic effect. In the greyhound, 4-aminopyridine also produced temporary cardiac arrhythmia which was only partly attributable to facilitated sympathetic transmission. In addition there was evidence of a central stimulant action of 4-aminopyridine and of a stimulant action on visceral activity. It is concluded that, while 4-aminopyridine may be useful in certain relatively rare conditions of neuromuscular transmission failure, its actions are too widespread for routine use as an antagonist to non-depolarizing neuromuscular blocking drugs.

4-Aminopyridine is used in Bulgaria, under the name “Pymadin” as an antagonist of non-depolarizing neuromuscular blocking drugs (Stoyanov and Vulchev, 1975; Stoyanov et al., 1976). Interest in the compound has recently become widespread, since it has been shown to facilitate neuromuscular transmission in the Eaton—Lambert syndrome (Lundh, Nilsson and Rosen, 1977; Agoston et al., 1978), in myasthenia gravis (Lundh, Nilsson and Rosen, 1979), in human botulism (Ball et al., 1979), and in the uncertain type of block produced by a combination of neuromuscular blocking drugs and some antibiotics (Booij, Miller and Crul, 1978). 4-Aminopyridine is devoid of anticholinesterase activity and acts by increasing the amount of acetylcholine released by nerve impulses. For a brief but comprehensive review of its actions, an article by Thesleff (1980) may be consulted.

The facilitatory actions of 4-aminopyridine on transmission are not confined to the neuromuscular junction. Transmission at autonomic adrenergic (Johns et al., 1976; Kirpkekar, Kirpkekar and Prat, 1977; Leander, Arner and Johansson, 1977; Glover, 1978) and cholinergic junctions, including sympathetic ganglia (Vizi, van Dijk and Foldes, 1977; Al-Haboubi et al., 1978; Moritoki, Takei, Nakamoto and Ishida, 1978; Durant, Lee and Katz, 1980), and at central synapses (Lemeignan, 1972; Jankowska et al., 1977; Folgering, Rutten and Agoston, 1979; Tokunaga, Sandri and Akert, 1979) is also affected. If 4-aminopyridine were to gain widespread use in man, full knowledge of its cardiovascular actions would be essential. Studies have been made on isolated blood vessels (Leander, Arner and Johansson, 1977; Glover, 1978) and cardiac tissues (Sobek, 1970; Lemeignan et al., 1975; Frank, Flom and Ffrench-Mullen, 1978; Yanagisawa and Taira, 1979; Freeman, 1979; Glover, 1979), but little information has been available about its effects on the intact cardiovascular system. For this reason, the experiments described in this paper were undertaken to study its cardiovascular actions in vivo, in anaesthetized cats and greyhounds.
MATERIALS AND METHODS

Cats

Cats of either sex were anaesthetized with a mixture of α-chloralose (80 mg kg⁻¹ in a 1% w/v solution) and sodium pentobarbitone (6 mg kg⁻¹) injected i.p. The trachea was intubated and positive pressure artificial ventilation was applied if it became necessary. The tendon of insertion of the tibialis anterior muscle was separated and detached, and connected to a Grass (model FT 10C) force transducer after clamping the limb in a horizontal position by means of drills through the femur and the tibia and fibula. Muscle temperature was maintained at 36–38 °C by means of lamps and a heated blanket; the temperature was monitored with a temperature probe (Grant Instruments, Cambridge). The sciatic nerve was ligated and cut high in the popliteal space and contractions of the tibialis anterior muscle were evoked by stimulating the trunk of the sciatic nerve after sectioning the main branches to other muscles. Rectangular stimulating pulses of 100|s duration and strength about double that necessary to evoke a maximal twitch were applied through platinum stimulating electrodes. The resting tension on the muscle was adjusted to that giving the greatest evoked twitch tension.

Arterial pressure was recorded from a femoral artery by means of a Statham (model P23AC) pressure transducer, and heart rate was recorded by a Grass (model 794C) tachograph triggered by the arterial pulse. The right vagus in the neck was exposed, tied centrally, and stimulated (rectangular pulses of 0.5 ms duration) through bipolar platinum electrodes at a frequency of 5–10 Hz (constant in any one experiment) for 5 s in every 100 s. The response to vagal stimulation was recorded as sharp decreases in both heart rate and arterial pressure.

The cervical sympathetic trunk on the right side was exposed and severed and the peripheral portion was stimulated (preganglionically) through bipolar platinum electrodes. The stimuli were rectangular pulses of 0.5 ms duration and of a strength greater than that necessary to evoke a maximal contraction of the nictitating membrane at the frequency of stimulation used. The pattern of stimulation applied was 5 Hz for 10 s every 100 s. The nictitating membrane on the same side (right) was attached to a Grass (model FTO3C) force transducer. The left nictitating membrane was similarly attached to a force transducer and stimulated through its postganglionic fibres with the same pattern of stimulation. The cervical sympathetic trunk was severed and followed cephalad until the nodose ganglion could be separated from the superior cervical ganglion, which was then crushed. A bipolar shielded hook electrode was placed so that the cathode was in contact with the postganglionic trunk and the anode with the crushed superior cervical ganglion. The contractions of the two membranes were similar in size. At the end of the experiments, an i.v. dose of hexamethonium 5 mg kg⁻¹ blocked the contractions of the preganglionically stimulated membrane (right), but did not block those of the postganglionically stimulated contralateral membrane, confirming that the stimulation on that side was in fact postganglionic.

All stimuli (tibialis anterior, vagus, pre- and postganglionic cervical sympathetic) were applied from Grass (model S4) stimulators, the stimuli being passed through Grass (model S1U5) isolation units. All records (arterial pressure, heart rate, contractions of the nictitating membranes to pre- or postganglionic stimulation, and contractions of a tibialis anterior muscle) were recorded concurrently on a Grass (model 7WC12PA) six-channel polygraph.

Greyhounds

Greyhounds of either sex and weighing between 22 and 31 kg were anaesthetized with thiopentone sodium 20 mg kg⁻¹ i.v. After endotracheal intubation, respiration was applied from a positive-pressure ventilation pump (25 strokes min⁻¹) with 100% oxygen. The stroke volume of the pump was adjusted to maintain an arterial \( P_{\text{CO}_2} \) of 35–40 mm Hg. Reflex movements were prevented by the intermittent i.v. administration of pancuronium bromide 4 mg and anaesthesia was maintained with α-chloralose 90 mg kg⁻¹ i.v. Under fluoroscopic control, radioopaque catheters were positioned in the descending aorta for the measurement of arterial pressure and for arterial blood sampling, the pulmonary artery for the measurement of pressure and for the determination of cardiac output by thermodilution and the coronary sinus for the sampling of venous blood draining from the heart. In addition, a catheter-tip transducer (Miller Instruments Inc., Houston, Texas) was introduced into the lumen of the left ventricle (via a carotid artery) for the measurement of left ventricular pressure and \( dP/dr \)
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The frequency response of this transducer system is flat to 200 Hz. Records of left ventricular pressure at high gain allowed accurate assessments to be made of left ventricular end-diastolic pressure (LVEDP).

After a left thoracotomy, the antero-lateral aspect of the heart was exposed and a calibrated electromagnetic flow probe (1.5–2.5 mm diameter) placed snugly around the left circumflex coronary artery for the measurement of coronary blood flow using a Statham SP2202 flowmeter with non-occlusive zeroing facilities. Positive end-expiratory pressure (2 cm H₂O) was initiated in all animals after thoracotomy to minimize alveolar shunting. Blood samples (1 ml) were regularly taken from the aorta, the coronary sinus and the pulmonary artery, and were analysed for oxygen and carbon dioxide tension and pH, using an IL blood-gas analyser. Haemoglobin concentrations were measured using a standard co-oximeter calibrated for greyhound blood. Myocardial oxygen extraction and consumption were calculated as outlined by Marshall and Parratt (1973).

Systemic arterial pressure (pulsatile and mean by electronic integration), pulmonary artery pressure, left ventricular pressure, left ventricular dP/dr, left circumflex coronary blood flow and the electrocardiogram (standard limb lead II) were recorded on an eight-channel ink-jet writing recorder (Mingograph 81).

Drugs and solutions

The drugs used were α-chloralose (Sigma), sodium pentobarbitone (Abbott), sodium thiopentone (May and Baker), 4-aminopyridine (Aldrich), atropine sulphate (British Drug Houses), hexamethonium bromide (May and Baker), phentolamine methylsulphonate (Ciba), propranolol hydrochloride (Imperial Chemical Industries), practolol (Imperial Chemical Industries), pancuronium bromide (Organon). They were dissolved in 0.9 w/v sodium chloride solution and the doses quoted refer to the bases or the cations.

Statistical analysis

Statistical analysis of the differences between means was performed using Student’s t test. P values of less than 0.05 were regarded as being significant.

RESULTS

Cats

In doses of 0.4 mg kg⁻¹ and greater, 4-aminopyridine produced a transient decrease followed by a sustained increase in arterial pressure and increases in the bradycardia response to vagal stimulation and in the contractions of the nictitating membrane evoked by pre- or postganglionic stimulation. At the same time, the twitches of the tibialis anterior muscle were increased in amplitude. This last response has been described elsewhere (Bowman, Rodger and Savage, 1979); it is mainly a consequence of a direct action on muscle contractility, but the well-known facilitatory action on neuromuscular transmission may contribute. The muscle record was included so that changes in other functions could be related to this more familiar response. The initial transient decrease in arterial pressure produced by 4-aminopyridine was not accompanied by bradycardia. The decrease was blocked by the previous injection of atropine 0.1 mg kg⁻¹. Figure 1 illustrates characteristic responses produced by 4-aminopyridine in doses of 0.4, 0.8 and 1.6 mg kg⁻¹ injected cumulatively (a total dose of 2.8 mg kg⁻¹).

Table I gives the mean results from this and four similar experiments. The highest cumulative dose used (2.8 mg kg⁻¹) caused cardiac irregularities including missed beats (fig. 1).

The fact that nictitating membrane responses to pre- and postganglionic stimulation were similarly augmented indicates an effect at the peripheral neuroeffector junction, but does not exclude an additional effect (masked in this type of experiment) at the superior cervical ganglion. In fact, Durant, Lee and Katz (1980) have recently shown that 4-aminopyridine does facilitate transmission through the superior cervical ganglion, and as a consequence it antagonizes ganglion block produced by hexamethonium, tubocurarine or polymyxin B. The results of Durant, Lee and Katz (1980) with hexamethonium were confirmed in two of the present experiments. Partial ganglion block was produced with hexamethonium 3 mg kg⁻¹. This was expressed as decreases in arterial pressure (of 80 and 110 mm Hg in the two cats) and 85–96% inhibition of the bradycardia response to vagal stimulation and of the nictitating membrane response to pre- but not to postganglionic stimulation. 4-Aminopyridine 1 mg kg⁻¹ rapidly (within 5 min) restored all responses to the control values; the responses then continued.
Table 1. Mean effects (± SEM) of 4-aminopyridine on the cardiovascular system, the nictitating membrane and the tibialis anterior twitch in five cats. *Mean of two results only; † mean of four results.

<table>
<thead>
<tr>
<th>Cumulative dose (mg kg⁻¹)</th>
<th>Arterial pressure (mm Hg)</th>
<th>Transient initial decrease in mean AP (mm Hg)</th>
<th>Heart rate (beat min⁻¹)</th>
<th>Vagal-induced decrease in mean AP (mm Hg)</th>
<th>Vagal-induced decrease in mean AP (mm Hg)</th>
<th>Nict. memb. (pre) (N)</th>
<th>Nict. memb. (post) (N)</th>
<th>Tibialis anterior twitch (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>96 ± 11.9</td>
<td>23 ± 3.4</td>
<td>101 ± 4.5</td>
<td>44 ± 3.0</td>
<td>53 ± 3.0</td>
<td>0.08 ± 0.007</td>
<td>0.06 ± 0.005</td>
<td>8 ± 0.7</td>
</tr>
<tr>
<td>0.4</td>
<td>114 ± 8.8</td>
<td>26 ± 3.4</td>
<td>106 ± 3.6</td>
<td>57 ± 4.6</td>
<td>66 ± 5.2</td>
<td>0.09 ± 0.009</td>
<td>0.08 ± 0.007</td>
<td>9.4 ± 0.9</td>
</tr>
<tr>
<td>1.2</td>
<td>130 ± 9.5</td>
<td>22 ± 3.4</td>
<td>122 ± 3.3</td>
<td>60* ± 4.6</td>
<td>79* ± 7.8</td>
<td>0.11 ± 0.008</td>
<td>0.10 ± 0.008</td>
<td>12 ± 0.9</td>
</tr>
<tr>
<td>2.8</td>
<td>141 ± 7.8</td>
<td>22 ± 2.5</td>
<td>122 ± 4.1</td>
<td>60* ± 4.1</td>
<td>79* ± 7.8</td>
<td>0.12† ± 0.01</td>
<td>0.11† ± 0.009</td>
<td>15 ± 1.5</td>
</tr>
</tbody>
</table>

4-AP (mg kg⁻¹ i.v.)

Fig. 1. Cat: chloralose anaesthesia. Records of arterial pressure (AP), heart rate, contractions of the right nictitating membrane in response to preganglionic stimulation at 5 Hz (Nic pre), contractions of the left nictitating membrane in response to postganglionic stimulation at 5 Hz (Nic post), and maximal twitches of a tibialis anterior muscle evoked by stimulation of its motor nerve at 0.1 Hz (Tib). The depressor and bradycardia responses were evoked by stimulation of the right vagus at 5 Hz for 5 s every 100 s. This was not possible throughout the final panel because of the convulsions of the animal. The gaps in the records correspond to 10 min.

4-Aminopyridine in cumulative doses up to 2.8 mg kg⁻¹ administered as in figure 1 did not affect the arterial pressure or nictitating membrane responses to acetylcholine 0.2 μg kg⁻¹ or adrenaline 1 μg kg⁻¹.

Even the smallest doses of 4-aminopyridine slowly to increase in amplitude in the manner illustrated in figure 1. The responses of the nictitating membrane to postganglionic stimulation were not affected by hexamethonium, but were increased in amplitude by 4-aminopyridine after the manner illustrated in figure 1.
injected (0.4 mg kg\(^{-1}\)) produced evidence of central stimulation (muscle twitching and eye movements). With increase in dose, these became more vigorous and interfered with the recordings. Thus, after a total dose of 2.8 mg kg\(^{-1}\) in the experiment illustrated in figure 1, twitches of the head, and generalized, though weak, clonic convulsions prevented further vagal stimulation and caused furri
ness of the nictitating membrane recordings. This was a consistent finding.

**Greyhounds**

4-Aminopyridine was studied at two doses—0.5 and 2.0 mg kg\(^{-1}\) i.v. As in the cat, the initial effect of 4-aminopyridine, occurring within 10 s of injection, was to produce a transient decrease (about 50 mm Hg) in arterial pressure which lasted about 30 s. The decrease was blocked by atropine 60 µg kg\(^{-1}\). Again, this decrease was not a consequence of a decrease in heart rate. It gave rise to secondary decreases in left ventricular pressure and in coronary blood flow as illustrated in figures 2 and 3.

The secondary, more slowly developing effect of 4-aminopyridine was a dose-dependent increase in arterial pressure and in left ventricular \(dP/dt\) max (figs 2, 3 and 4). (Each value in figure 4 and in the following description is the mean ± SEM.) There was also a significant increase in coronary blood flow (from 21 ± 6 to 36 ± 7 ml min\(^{-1}\); \(P < 0.05\)), an increase in stroke volume especially after the larger dose (2 mg kg\(^{-1}\)), and a small, but statistically significant increase in heart rate after the smaller dose (0.5 mg kg\(^{-1}\)) only. In accordance with the increase in arterial pressure, there was an increase in left ventricular systolic pressure, but no significant change in left ventricular end-diastolic pressure (4 ± 1 mm Hg before and 4 ± 2 mm Hg after 4-aminopyridine). There was a slight increase in right atrial pressure and in mean pulmonary artery pressure (9 ± 3 to 14 ± 2 mm Hg). These changes in cardiovascular parameters reached their peaks 10–20 min after injection; they had usually returned to control by 40–50 min after injection of 2 mg kg\(^{-1}\), although in two dogs the arterial pressure remained increased for more than 80 min, at which time the experiment was terminated. The main changes in cardiovascular parameters are illustrated in figures 2, 3 and 4.

Although oxygen extraction by the heart was reduced to a small extent (51 ± 1 to 43 ± 4%; \(P < 0.05\)), myocardial oxygen consumption was significantly increased (from 2.8 ± 0.9 to 3.6 ± 0.8 ml 100 g\(^{-1}\) min\(^{-1}\)) due to the increase in arterial pressure, and thus in left ventricular pressure and coronary blood flow.

**Fig. 2.** Greyhound: sodium thiopentone–chloralose anaesthesia. Effects of 4-AP 0.5 mg kg\(^{-1}\) on e.g., arterial pressure (AP), right atrial pressure (RAP), pulmonary artery pressure (PAP), left ventricular pressure (LVP), left ventricular \(dP/dt\) (LV\(dP/dt\)), and coronary artery blood flow (CBF). Records were made at two different paper speeds as indicated by the time calibrations.
4.6 ± 1.1 ml min⁻¹; P < 0.01) as a consequence of increased myocardial blood flow and external cardiac work (1.7 ± 0.3 to 3.4 ± 0.4 kg m⁻¹min⁻¹; P < 0.05). Blood haemoglobin increased by about 3.8% (P < 0.01) after 4-aminopyridine 2 mg kg⁻¹, presumably as a consequence of contraction of the spleen. In a dose of 2 mg kg⁻¹, 4-aminopyridine consistently increased arterial Pco₂ (from 35 ± 2 to 39 ± 2 mmHg; P < 0.05) and increased arterial oxygen content, because of the increase in haemoglobin, from 26.8 ± 4 to 29.8 ± 2 ml dl⁻¹. Arterial pH was not significantly affected by 4-aminopyridine, being 7.37 ± 0.02 units before and 7.37 ± 0.03 units 20 min after injection of 2 mg kg⁻¹.

Pretreatment with practolol in a dose (1 mg kg⁻¹) sufficient to produce about a 20-fold shift of the dose-chronotropic response curve to isoprenaline prevented the increases in stroke volume, in heart rate, in dP/dt max and in coronary blood flow produced by 4-aminopyridine. After practolol, 4-aminopyridine produced a slight increase in left ventricular end-diastolic pressure (10 ± 2 mm Hg before and 14 ± 2 mm Hg after 4-aminopyridine in the presence of prac- tolol), and a small decrease in heart rate instead of a small increase. Surprisingly, practolol blocked the increase in mean arterial pressure, and in diastolic pressure, produced by 4-aminopyridine. The increase in arterial pressure produced by 4-aminopyridine was also blocked by phentolamine 1 mg kg⁻¹.

Pretreatment with atropine 60 μg kg⁻¹, in the absence of practolol, potentiated the tachycardia produced by 4-aminopyridine, but did not significantly modify the increases in arterial pressure, in stroke volume and in dP/dt max. The effects of practolol and atropine on responses to 4-aminopyridine are illustrated in the histograms of figure 4.

4-Aminopyridine, even in the smaller dose (0.5 mg kg⁻¹), produced changes in the configuration of the e.c.g. and occasional arrhythmic episodes. In the experiment illustrated in figure 2, 4-aminopyridine 0.5 mg kg⁻¹ produced an increase in the size of the P wave, and later a brief period of arrhythmia. In the experiment of figure 3, on the other hand, 4-aminopyridine 2 mg kg⁻¹ produced a pronounced increase in the T wave. This was a commonly occurring effect of 4-aminopyridine. The same dose also gave rise to occasional non-functional ectopic electrical responses, each followed by a compensatory pause. The QRS complex did not seem to be modified. Figure 5 illustrates enlarged e.c.g. records extracted from
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Fig. 4. Histograms illustrating the effects of 4-aminopyridine 0.5 and 2.0 mg kg$^{-1}$ on heart rate, stroke volume, mean arterial pressure and left ventricular dP/dt max of anaesthetized greyhounds. Controls are represented as white rectangles and responses to 4-aminopyridine as black rectangles. The effects of 4-aminopyridine 2.0 mg kg$^{-1}$ are shown before and after practolol 1 mg kg$^{-1}$ or atropine 60 μg kg$^{-1}$. Vertical lines indicate SEM. Asterisks indicate a significant difference from control (P<0.05).

an experiment similar to those illustrated in figures 2 and 3. The T wave is often small and inverted in lead II from anaesthetized greyhounds and this is so in the control (top) record of figure 5. Five minutes after a dose of 4-aminopyridine 2 mg kg$^{-1}$, the T waves were greatly increased and the P waves also enlarged. Forty-six minutes after injection there were ectopic responses with inverted P waves, suggesting that they were of high A-V nodal origin. Fifty-two minutes after injection there were large premature ectopic responses, probably of ventricular origin. They were regular in occurrence (three normal beats between each). The ectopic electrical responses were not associated with ventricular contractions. By 60–70 min after injection, the e.c.g. was invariably restored to the control appearance. The abnormal e.c.g. changes produced by 4-aminopyridine still occurred after treatment with practolol 1 mg kg$^{-1}$, but were less pronounced and appeared to revert more quickly to sinus rhythm.

In every experiment, a dose of 4-aminopyridine 2 mg kg$^{-1}$ produced clear evidence of additional somatic and autonomic effects. In spite of the administration of pancuronium, there were marked movements of the limbs and diaphragm. These effects probably reflected a central stimulant action which was powerful enough to “break through” the peripheral neuromuscular block. In addition, the animals exhibited one or more of the following: retching, defaecation, micturition, salivation, and disgorgement of bile into the oesophagus. There was also a small but transient increase in oesophageal temperature.

DISCUSSION

In the cat, 4-aminopyridine augmented the bradycardia produced by vagal stimulation and the contractions of the nictitating membrane produced by pre- or postganglionic stimulation without changing the responses produced by acetylcholine or adrenaline. These effects are compatible with the observations made by others on isolated tissues (see Introduction for references) that 4-aminopyridine facilitates transmission at autonomic synapses and neuro-effector junctions, as well as at the neuromuscular junction in skeletal muscle. They further show that these autonomic effects are produced by doses little different from those required to facilitate neuromuscular transmission. The fact that nictitating membrane responses to postganglionic stimulation were augmented suggests that 4-aminopyridine facilitates noradrenaline release from the cervical sympathetic nerves. The effect was not great (approx. plus 50%), but in one preliminary experiment a much greater augmentation (>600%) of contractions evoked by postganglionic stimulation was produced when the responses had been depressed to 16% of control by the α-adrenoceptor blocking drug phentolamine (2 mg kg$^{-1}$). It thus appears that facilitation of noradrenergic transmission is more obvious when that transmission is previously impaired. The same is true for neuromuscular transmission in skeletal muscle, and for ganglionic transmission as demonstrated by Durant, Lee and Katz (1980) and confirmed here. The experiments
provide no evidence as to whether the facilitation of vagal transmission occurred at the vagal ganglia or at the neuro-effector junctions in the S–A node of the heart, but by analogy with other sites it probably occurred at both.

In so far as the heart is concerned, presumably the increased vagal influence on the S–A node (perhaps further augmented reflexly as a consequence of the increase in arterial pressure) partially counteracts the opposite effect arising from facilitation of sympathetic transmission, so that the change in resting heart rate (a small positive chronotropic effect) was slight in both the cat and the dog. Evidence in support of this interpretation is provided by the observation that atropine, presumably by removing the vagal opposition, increased the positive chronotropic effect of 4-aminopyridine, whereas practolol converted it to a bradycardia. 4-Aminopyridine produced a substantial positive inotropic effect, evident in the dog as an increase in left ventricular dP/dt max and in stroke volume. That this effect was the result of facilitation of sympathetic transmission was strongly suggested by the fact that it was blocked by practolol. Since the vagal innervation of ventricular muscle is sparse, little counteraction of the sympathetic effect on the ventricles would occur. Evidence from the use of isolated cardiac tissues...
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(Freeman, 1979; Glover, 1979; Yanagisawa and Taira, 1979) suggests that 4-aminopyridine may exert a direct positive inotropic effect. However, the doses used in vivo in our experiments were probably not large enough to evoke such an action. In any case, there is controversy about the existence of such a direct effect. According to Rodger and Shahid (1981) the direct effect in isolated cardiac muscle is largely a non-specific consequence of the increased pH produced by 4-aminopyridine; these authors observed that, when neutral pH is maintained, the direct cardiac effect of 4-aminopyridine is trivial. 4-Aminopyridine does not, of course, produce detectable pH changes in vivo because of the pronounced buffering capacity of the blood.

The phentolamine-sensitive increase in diastolic pressure produced by 4-aminopyridine can also be attributed to facilitation of sympathetic drive, both to the blood vessels and to the spleen, and most of the remaining haemodynamic effects (increased coronary flow, increased myocardial oxygen consumption, increased haemoglobin and blood-gases) can probably be considered as secondary to the increased arterial pressure and cardiac performance. It is, however, not clear why practolol (as well as phentolamine) should have blocked the increase in diastolic pressure produced by 4-aminopyridine in the greyhound and no explanation can be offered.

The mechanism underlying the initial transient atropine-sensitive decrease in arterial pressure produced by 4-aminopyridine is also unclear. It was not secondary to a bradycardia and was therefore a consequence of vasodilatation. It seems unlikely that 4-aminopyridine should directly stimulate muscarinic receptors, and this points to the involvement of acetylcholine. Yet the transient nature of the effect is unlike any other facilitatory effect of 4-aminopyridine at a site of synaptic or neuro-effector transmission. Possibly a bolus injection of 4-aminopyridine briefly stimulates some afferent nerve endings that reflexly activate cholinergic vasodilator fibres, but the effect requires further study before any definite conclusion can be reached.

The e.c.g. abnormalities produced by 4-aminopyridine are probably explicable, at least partly, in terms of interactions between simultaneously facilitated adrenergic and cholinergic transmission to the heart and the known ability of the compound to block potassium channels in excitable membranes (Pelhate and Pichon, 1974) including those of the heart (Freeman, 1979). The observation that the arrhythmia was reduced in extent by practolol suggests a sympathetic component, but the fact that they were not abolished indicates this to be only one facet of the arrhythmogenic action. The arrhythmias are in fact somewhat reminiscent of those associated with hyperkalaemia, and the possible ability of 4-aminopyridine to increase blood potassium ion concentration (possibly by facilitating adrenergic transmission to the liver) is therefore worth investigating.

The obvious question arises as to the clinical significance of the observations made in animals. There are probably enough data available from the use of 4-aminopyridine in man to suggest that the results of these animal experiments exaggerate the potential side-effects. The anaesthetized greyhound, for example, is known to be unusually susceptible to cardiac arrhythmia (Marshall and Parratt, 1973). In addition, the doses used in the animal experiments are somewhat greater than those usually used clinically. However, Ball and his co-workers (1979) who administered, to patients with botulism, doses of 4-aminopyridine not greatly different from those used in the animal experiments, recorded increases in arterial pressure, increases in heart rate, gastrointestinal stimulation and convulsive effects. It therefore seems that 4-aminopyridine may not be totally suitable for routine use in anaesthetic practice as an antagonist to neuromuscular blocking drugs. Rather, its use should perhaps be restricted to rare conditions of transmission failure (Eaton–Lambert syndrome, botulism, block involving certain antibiotics) that do not respond well to other measures. Obviously, its safety would be increased were it to be used in relatively small doses to potentiate other antagonists, such as neostigmine, after the manner demonstrated by Miller and colleagues (1979) and Ball and others (1979). The possibility that the cardiovascular effects of 4-aminopyridine might be exploited in circulatory shock conditions should perhaps also be explored in animals, since it has the unusual action of facilitating the body's natural reflex efforts to restore the circulation.

Although 4-aminopyridine itself may not be suitable for development as a drug for routine use, its novel and potent actions are worthy of further study. Furthermore, it is possible that analogues may be found that are more selective in their...
actions, with a consequent reduction in their tendency to produce side-effects.

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On procédé à l'étude des effets de l'agent anti-curare aminopyridine-4 sur les systèmes cardiovasculaires de chats et de lévriers anesthésiés au chloralose barbiturique. Dans ces deux espèces, l'aminopyridine-4 occasionnait une baisse temporaire de sensibilité à l'atropine de la tension artérielle, suivie d'une hausse prolongée de caractère adrénergique. Chez le chat, les réponses cardiaques à la stimulation vagale et les réponses de la membrane clignotante à la stimulation lymphatique se sont accrues après l'injection d'aminopyridine-4 et il devint clair que ces effets étaient dus à la libération croissante de neuro-
transmetteurs. Chez le lévrier, l’aminopyridine-4 provoque des augmentations de la pression systolique du ventricule gauche et du max dP/dt, de la pression atriale droite, du volume systolique, du courant sanguin myocardique, de la consommation d’oxygène myocardique, de la fonction cardiaque externe, du contenu d’oxygène artériel et de l’hémoglobine du sang. Ces effets peuvent être attribués à la plus grande facilité de transmission sympathique aux vaisseaux sanguins, au cœur et à la rate. Le rythme cardiaque ne fut pas grandement affecté, car la facilité de transmission vagale au nodus S-A contraintrait l’effet sympathique accru. Chez le lévrier, l’aminopyridine-4 provoqua également une arythmie temporaire qui n’était que partiellement attributable à la transmission sympathique plus aisiée. En outre, des signes évidents d’une action stimulante centrale de l’aminopyridine-4, ainsi qu’une action stimulante sur l’activité viscérale, se manifestèrent. On en tire la conclusion que, nonobstant l’utilité de l’aminopyridine-4 dans certains cas relativement rares d’insuffisance de transmission neuromusculaire, ses effets sont beaucoup trop dispersés pour en user d’office comme antagoniste à l’égard de substances de blocage neuromusculaire non dépolarisantes.

WIRKUNGEN VON 4-AMINOPYRIDIN AUF DIE HERZGEFASS-SYSTEME VON ANASTHETIIERTEN KATZEN UND HUNDEN

ZUSAMMENFASSUNG


ACCIONES DE LA AMINOPIRIDINA-4 SOBRE SISTEMAS CARDIOVASCULARES DE GATOS Y PERROS ANESTESIADOS

SUMARIO

Se estudió los efectos del agente anticurare aminopiridina-4 sobre los sistemas cardiovasculares de gatos y perros galgos anestesiados con cloralosa barbiturica. En estas dos especies, la aminopiridina-4 provocó una disminución transitoria de sensibilidad atropínica de la presión arterial, seguida por un aumento prolongado de la misma obtenida por medio adrenérgico. En el gato, las respuestas cardiacas al estímulo vagal y las de la membrana nictitante al estímulo linfático aumentaron después de la inyección de aminopiridina-4 y se puso de manifiesto que dichos efectos se debían a la liberación creciente de neurotransmisores. En el perro galgo, la aminopiridina-4 causó aumentos de la presión sistólica del ventrículo izquierdo y del máximo. dP/dt, de la presión atrial derecha, del volumen sistólico, de la corriente sanguínea miocárdica, del consumo de oxígeno miocárdico, de la función cardíaca externa, del contenido arterial de oxígeno y de la hemoglobina de la sangre. Se pueden atribuir estos efectos a la facilitación de la transmisión simpática a los vasos sanguíneos, al corazón y al bazo. No fue afectado mayormente el ritmo cardíaco, puesto que la facilitación de la transmisión vagal al nodo S-A contrarió el efecto simpático creciente. En el perro galgo, la aminopiridina-4 ocasional también una arritmia pasajera que podía atribuirse parcialmente solamente a la transmisión simpática facilitada. Además, se puso de manifiesto la acción estimulante central de la aminopiridina-4, así como su acción estimulante sobre la actividad visceral. Se llega a la conclusión de que, a pesar de la utilidad de la aminopiridina-4 en ciertos casos relativamente excepcionales de deficiencia de transmisión neuromuscular, sus efectos son demasiado diseminados como para usarla de rutina con el fin de antagonizar a las substancias de bloqueo neuromuscular no-depolarizantes.