EFFECT OF 4-AMINOPYRIDINE ON CARDIOVASCULAR FUNCTIONS IN THE DOG*

É. MARTÍNEZ-AGUIRRE, J.A. WIKINSKI, A. BELLO, J. IZQUIERDO, A. GARCIA AND HILDA VELARDE

ABSTRACT

The drug 4-aminopyridine (4-AP) at a dose of 0.2 mg · kg^{-1} body weight intravenously is an effective antagonist of non-depolarizing neuromuscular blockade. We studied its cardiovascular effects in the canine heart using a right-heart bypass with extracorporeal circulation in seven dogs. This study demonstrates that 4-aminopyridine significantly augments arterial blood pressure, left ventricular dp/dt maximum, as well as left ventricular pressure at dp/dt mx. The highest values were obtained between two and 20 minutes after administration of 4-AP. Left ventricular end-diastolic pressure diminished slightly, but this was not statistically significant. Although the exact mechanism of action of 4-AP is not known, its positive inotropic effects may be of value in the reversal of non-depolarizing neuromuscular blockade in patients with impaired myocardial function of diverse aetiologies and it would be contraindicated in patients with arterial hypertension and/or coronary artery disease.

KEY WORDS: ANTAGONISTS, NEUROMUSCULAR RELAXANTS; 4-aminopyridine, cardiovascular effects.

THE DRUG 4-AMINOPYRIDINE (4-AP) is a simple heterocyclic chemical compound. Its convulsant properties in the frog were described in 1928 by Dingemanse and Wibaut.[1] There have been several reports of its facilitatory action on transmission at various synapses and neuroeffector junctions, both in vertebrates and invertebrates.[2-7,9] Furthermore, there have been reports of the augmentation of skeletal muscle contractility by its direct action on the muscle fibre.[2,3,8] Although it did not have a direct anticholinergic activity, it was suggested that it facilitates neuromuscular transmission by increasing acetylcholine release.[9,10]

The use of 4-AP hydrochloride (Pimadin, Pharmachin Laboratory), as an anticurare agent in clinical anaesthesia has been described[11-13] with some reported effects on the cardiovascular system.[14] Atropine is not required and spontaneous ventilation is restored better and for a longer period than by anticholinesterase drugs. In addition, it was stated that this drug could be safely administered to debilitated and critically ill patients, including those with cardiac disease.[11] However, since no systematic evaluation of its direct myocardial actions has been reported, we tested the drug in the isolated heart preparation of the dog, as previously described by our research group.[16]

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MATERIAL AND METHOD

Seven healthy mongrel dogs weighing approximately 25 kg each were anaesthetized with sodium thiopentone one percent intravenously in a total dose of 20 to 30 mg · kg^{-1} body weight. Anaesthesia was maintained with nitrous oxide and oxygen (2:1 mixture), and d-tubocurare was administered in small doses to ensure adequate muscle relaxation without causing noticeable cardiovascular effects. If necessary, additional doses of 25 to 50 mg of thiopentone were administered to ensure unconsciousness of the dog during the procedure.

The trachea was intubated with a cuffed rubber tracheal tube, and ventilation was controlled with a mechanical volume-limited ventilator adjusted to maintain Pa_{O_2}, Pa_{CO_2} and pH within physiological limits. Anticoagulation was achieved with sodium heparin 3 mg · kg^{-1} administered intravenously at hourly intervals.

A right heart bypass (Figure 1) was used in all

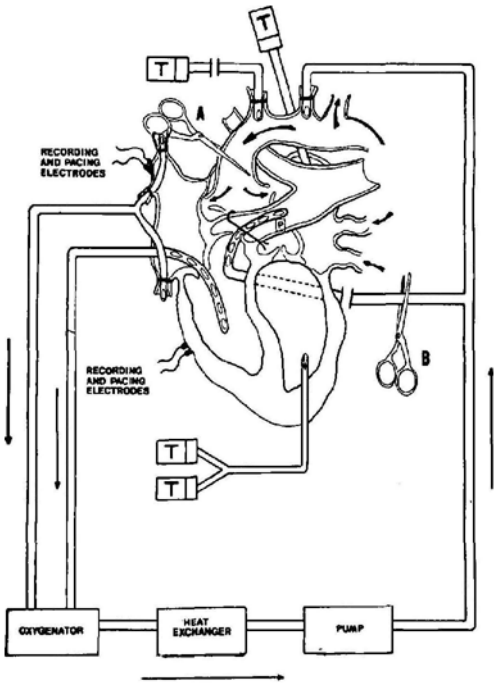

FIGURE 1 Right heart bypass preparation. T: Pressure Transducer, P: Tip of the cannula in the pulmonary artery. Note that main flow is preserved in the right heart bypass preparation. The arrows indicate the direction of blood flow. Left ventricular systolic and diastolic pressures were measured by means of a short "Y" shaped metal cannula introduced into the left ventricle. The clamps A and B are only used for vascular resistance studies.

animals. This preparation serves to control cardiac input (preload) in an otherwise normally functioning left heart preparation. The chest was opened through a median sternotomy and an extracorporeal circulation system was connected. The venous return from the cannulated superior and inferior venae cavae was directed to a reservoir and the coronary venous effluent was drained by gravity from the non-working right heart into the same reservoir. The oxygenated blood in the reservoir was then led to a heat exchanger to be warmed to 37°C, and subsequently returned to the pulmonary artery by an occlusive roller pump. Cardiac input (preload), which ultimately determines left ventricular output, remained constant during the experiments. The flow was maintained constant at values ranging between 80 and 120 $ml \cdot kg^{-1} \cdot min^{-2}$.

Intravascular pressures were measured in the aorta through the carotid artery with a Hewlett-Packard 1280 strain-gauge transducer. Left ventricular systolic, diastolic and mean pressures were measured by means of a Y-shaped metal cannula introduced into the left ventricle through a small incision in its tip, and also attached to Hewlett-Packard 1280 strain-gauge transducers. The pressure measurements showed a phase shift which was linear from 0 to 30 Hz. The damping coefficient of the pressure measurement system was 1.0 at a time constant of 0.1 second. The rate of increase of left ventricular pressure (LV dp/dt), was computed electronically using a Hewlett-Packard 8800 derivative computer. Filters were used in the derivative computer to operate between 30 and 100 Hz. This range has proved reliable for registering changes in the contractile state of the heart. The electrocardiogram was obtained using recording electrodes for standard bipolar derivations. The circulatory parameters were registered continuously, and high-speed (100 mm/min) tracings were obtained at zero time, and at two, four, 20 and 30 minutes after the injection of 4-AP (Figure 2).

Seven dogs were treated with 4-AP 0.2 $mg \cdot kg^{-1}$, which is the usual clinical dose for

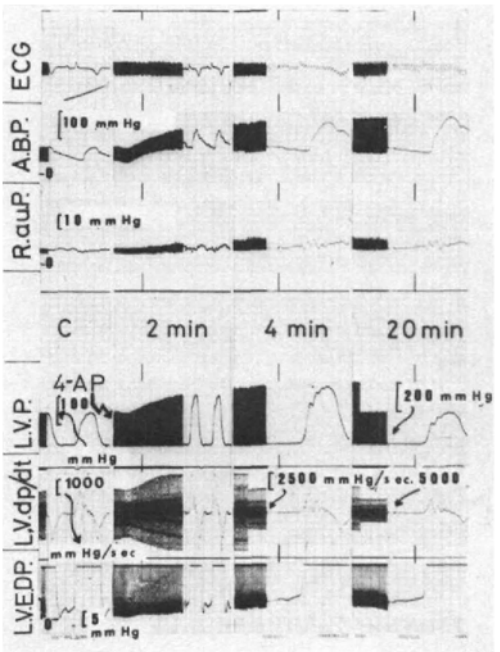

FIGURE 2 ECG: electrocardiogram D II, ABP: arterial blood pressure, R au. P: right auricular pressure, LVP: left ventricular pressure, LVdp/dt: left ventricular dp/dt, LVEDP: left ventricular end-diastolic pressure. Tracing sampled at control (C), 2, 4, 20 and 30 minutes after administration of 4-aminopyridine. Note the increment of ABP, LVP and LVdp/dt that begins 2 minutes after 4-AP administration (see text for more details). [: Register sensitivity range.

TABLE I

ARTERIAL BLOOD PRESSURE VARIATIONS, EXPRESSED AS DELTA OF CONTROL VALUE, AT +2, +4, +20 AND +30 MINUTES AFTER ADMINISTRATION OF 4-AP 0.2 $mg \cdot kg^{-1}$ (N = 7)
(Time (Min) After Administration of 4-AP)

	+2		+4		+20		+30	
	S(°)	D(*)	S	D	S	D	S	D
	30	25	35	25	50	35	40	30
	15	0	30	25	35	35	50	50
	20	10	25	15	25	25	20	20
	15	10	40	15	75	35	85	25
	15	10	15	10	30	25	15	20
	10	10	20	20	30	25	25	35
	30	30	33	28	53	30	58	25
$\bar{X}$	19.3	13.6	28.3	19.7	42.6	30.0	41.9	29.3
S.D.	±7.9	±10.3	±8.8	±6.6	±17.8	±5.0	±24.7	±10.6
S.E.	±2.98	±3.98	±3.32	±2.49	±6.72	±1.88	±9.32	±4.0

Control vs. all time intervals $P < 0.001$.
Systolic +2 vs. Systolic +4: N.S.
Diastolic +2 vs. Diastolic +4: N.S.
Systolic +4 vs. Systolic +20: N.S.
Diastolic +4 vs. Diastolic +20 $P < 0.01$.
Systolic and Diastolic +20 vs. +30: N.S.

(°) Systolic Blood Pressure.
(*) Diastolic Blood Pressure.
$\bar{X}$: Mean.
S.D.: Standard Deviation.
S.E.L: Standard Error of Mean.
N.S.: Not significant.

reversal of non-depolarizing neuromuscular block.[12]

The initial values of the cardiovascular data were compared with those obtained after drug administration and the results were evaluated through t-test for paired data for small groups.[26]

RESULTS

Variations of Systolic and Diastolic Arterial Blood Pressure

There was a significant increase in arterial blood pressures when the control values were compared with all the selected time intervals. The most elevated pressures obtained were registered at +20 and +30 minutes (Table I and Figure 3).

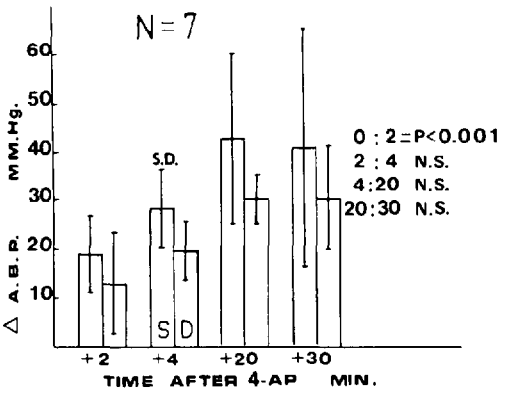

FIGURE 3 Variation in systolic (S) and diastolic (D) arterial blood pressure, with respect to control value 2, 4, 20 and 30 minutes after 4-AP administration. The increase in systolic and diastolic pressures were significant ($P < 0.001$) for all time intervals compared with control (0) value. Maximum increase in arterial blood pressure was observed at 20 minutes. ($\bar{X}$ = Mean ± S.D.)

Cardiac Rate

There were no significant variations of cardiac rate.

Left Ventricular Pressure (LVP)

There was a significant and prolonged increase of left ventricular pressure with the highest values registered at +20 and +30 minutes (Table II and Figure 4).

Left Ventricular Maximum dp/dt (LV dp/dt max.)

There was a significant increase in LV dp/dt max at +2, +4 and +20 minutes. Although the values were still elevated at +30 minutes, this increase was not statistically significant with respect to the +20 minute value (Table III and Figure 5).

TABLE II

LEFT VENTRICULAR PRESSURE VARIATIONS, EXPRESSED IN mmHg AS DELTA OF CONTROL VALUE AT +2, +4, +20, +30 MINUTES AFTER THE ADMINISTRATION OF 4-AP, 0.2 mg · kg^{-1} (N = 7)
(Time (Min) After the Administration of 4-AP)

	+2	+4	+20	+30
	20	25	90	30
	15	30	35	60
	10	15	14	13
	15	20	55	70
	10	28	60	55
	18	30	55	25
	22	22	53	60
$\bar{X}$	15.7	24.3	51.7	44.7
S.D.	±4.15	±5.6	±23.3	±21.7
S.E.	±1.74	±2.11	±8.79	±8.19

Control vs. all time intervals $P < 0.001$. $\bar{X}$: Mean
+2 vs. +4 $P < 0.01$. S.D.: Standard Deviation.
+4 vs. +20 $P < 0.025$. S.E.: Standard Error of mean.
+20 vs. +30 N.S. N.S.: Not significant.

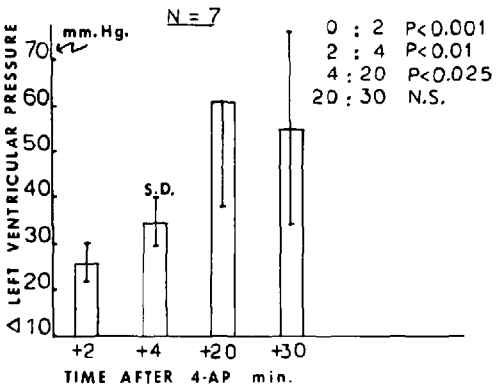

FIGURE 4 Variation of Left Ventricular Pressure (LVP) with respect to control value, 2, 4, 20 and 30 minutes after 4-AP administration. The increase in LVP was significant ($P < 0.0001$) for all time intervals compared with control value (0), and also comparing 2 and 4 minute intervals ($P < 0.01$) and 4 and 20 minute intervals ($P < 0.025$). Maximum increase was observed 20 minutes after 4-AP administration. ($\bar{\bar{X}}$ = Mean ± S.D.).

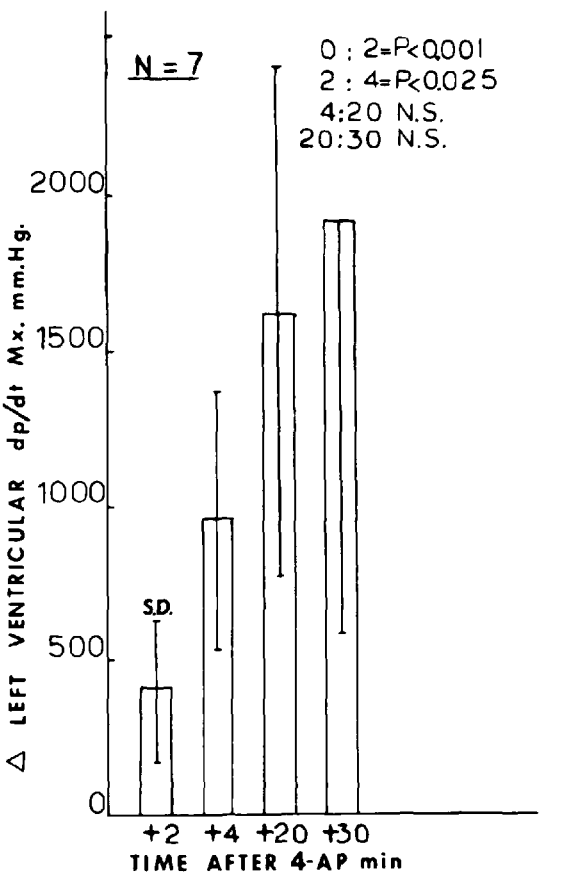

FIGURE 5 Variation of left ventricular dp/dt maximum (LV dp/dt mx) with respect to control value 2, 4, 20 and 30 minutes after 4-AP administration. The increase in LVdp/dt mx was significant ($p < 0.001$) for all time intervals compared with control value (0); and $P < 0.0025$ for 2 and 4 minute intervals. Maximum LVdp/dt mx increase was observed between 20 and 30 minutes after 4-AP administration. ($\bar{\bar{X}}$ = Mean ± S.D.).

TABLE III

LEFT VENTRICULAR dp/dt Mx VARIATIONS, EXPRESSED IN mmHg/sec, AS DELTA OF CONTROL VALUE, AT +2, +4, +20, +30 MINUTES AFTER THE ADMINISTRATION OF 4-AP 0.2 mg·kg^{-1} (N = 7)
(Time (Min) After the Administration of 4-AP)

	+2	+4	+20	+30
	300	500	600	−50
	700	1 000	2 100	2 800
	0	500	600	600
	500	1 400	1 900	2 100
	300	500	1 200	1 800
	600	1 500	2 200	2 200
	500	1 200	2 800	4 000
$\bar{X}$	414	957	1 629	1 921
S.D.	±234	±428	±846	±1 345
S.E.	±88.3	±161.5	±319.2	±507.6

Control vs. all time intervals	P < 0.001.	$\bar{X}$: Mean
+2 vs. +4	P < 0.025.	S.D.: Standard Deviation.
+4 vs. +20	N.S.	S.E.: Standard Error of mean.
+20 vs. +30	N.S.	N.S.: Not significant.

TABLE IV

LEFT VENTRICULAR PRESSURE AT dp/dt Mx VARIATIONS, EXPRESSED IN mmHg/sec, AS DELTA OF CONTROL VALUE, AT +2, +4, +20, +30 MINUTES AFTER THE ADMINISTRATION OF 4-AP 0.2 mg·kg^{-1} (N = 7)
(Time (Min) After the Administration of 4-AP)

	+2	+4	+20	+30
	10	30	30	5
	25	35	30	50
	20	20	20	20
	20	40	40	55
	10	25	38	25
	15	32	45	25
	25	40	40	35
$\bar{X}$	17.9	31.7	34.7	30.7
S.D.	±6.4	±7.5	±8.7	±17.4
S.E.	±2.42	±2.83	±3.21	±6.57

Control vs. all time intervals	P < 0.001.	$\bar{X}$: Mean
+2 vs. +4	P < 0.005.	S.D.: Standard Deviation.
+4 vs. +20	N.S.	S.E.: Standard Error of mean.
+20 vs. +30	N.S.	N.S.: Not significant.

Left Ventricular Pressure at Maximum dp/dt (LVP dp/dt max.)

There was a significant increase in LVP dp/dt max at all time intervals. Maximum values were registered at +20 minutes (Table IV and Figure 6).

Left Ventricular End-Diastolic Pressure (LVEDP)

After a brief non-significant initial increase at +2 minutes, there is a small decrease in left ventricular end-diastolic pressure which was not significant at all time intervals (Table V).

DISCUSSION

Anticholinesterase drugs are effective antagonists of nondepolarizing neuromuscular blocking agents. But due to undesirable muscarinic side effects (salivation, bradycardia, bronochospasm, etc.) other drugs have been employed, such as tetraethylammonium, germine diacetate, guanidine, and 4-AP. Even though an effective anticurare action has been reported for the latter drug,[8] only Lee *et al.*[14] have reported some cardiovascular effects.

TABLE V

LEFT VENTRICULAR END-DYASTOLIC PRESSURE VARAITIONS, EXPRESSED IN mmHg AS DELTA OF CONTROL VALUE AT +2, +4, +20, +30 MINUTES AFTER THE ADMINISTRATION OF 4-AP 0.2 $mg \cdot kg^{-1}$ (N = 7)
(Time (Min) After the Administration of 4-AP)

	+2	+4	+20	+30
	1	0	0	−5
	3	5	0	−2
	0	−5	−8	−5
	0	0.5	−3	−3
	0	0	−1	−3
	0	0	−2	−1
	−1	−2	−2	−2
$\bar{X}$	0.4	−0.2	−2.3	−3.0
S.D.	±1.3	±3.0	±2.8	±1.5
S.E.	±0.49	±1.13	±1.06	±0.57

Control vs. all time intervals	N.S.	$\bar{X}$: Mean
+2 vs. +4	N.S.	S.D.: Standard Deviation.
+4 vs. +20	N.S.	S.E.: Standard Error of mean.
+20 vs. +30	N.S.	N.S.: Not significant.

The isolated heart preparation previously described[16] is suitable for this type of evaluation. From our results, it is clear that 4-AP has a significant positive inotropic effect and produces a substantial and prolonged increase in arterial blood pressure. This is in contradiction to the observation of Paskov *et al.*,[11] who found no cardiovascular effects. The observation of a sustained and prolonged increment in arterial blood pressure and myocardial contractility produced by 4-AP suggest the possibility of its use for reversal of neuromuscular block in patients with some cardiac diseases with concomitant decrease in the mechanical function of the heart. Its use in patients with low-flow syndrome, in myocardial depression consecutive to the use of anaesthetic agents, and in shocked patients, may also be of interest. On the contrary, its use in patients with arterial hypertension and/or coronary artery disease would appear to be contraindicated. At the present time we do not know the exact mechanism of action of 4-AP. Therefore the exact mechanism of this positive inotropic effect is only speculative, as experimental evidence is lacking; although its main pharmacological actions (increased spontaneous and evoked transmitter release and increased muscle contractility) resemble the effect of calcium ions. So it may act by modifying calcium fluxes[17] or by selective blocking of potassium currents.[15,18-20] An intracellular action on calcium stores in muscle fibres therefore appears feasible. From the results of Lundh *et al.*[21,22] it seems reasonable to speculate that 4-AP may act by modifying intracellular calcium concentrations in the myocardial fibre. These authors have stated that such actions would represent a novel type of drug effect that could be of clinical importance, and it offers a method of value in electrophysiological studies of the role of calcium in evoked transmitter release.

Furthermore, 4-AP produces central nervous system stimulatory effects,[3] and an antagonizing effect to ketamine and benzodiazepines,[23] which can be actions additional to its cardiac inotropic effects. Similar effects have been described for tetrahydroaminoacridine (T.H.A.)[25] with which it may share structure/action relationship. We can speculate that in the future it may become a useful drug, available to the anaesthetist and the cardiologist for their clinical practice.

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RÉSUMÉ

En administration intraveineuse à la dose de 0.2 mg · kg^{-1}, la 4-aminopyridine (4-AP) est un antagoniste efficace du bloc neuromusculaire produit par les relaxants de type non-dépolarisant. Nous avons étudié ses effets cardiaques chez sept chiens sous circulation extracorporelle avec dérivation du cœur droit. Cette étude a montré que la 4-aminopyridine élève de façon significative la pression artérielle et la dp/dt maximale du ventricule gauche, les valeurs maximales étant obtenues entre deux et vingt minutes après l'injection du médicament. La pression de fin de diastole du ventricule gauche diminuait légèrement, mais de façon non significative. Bien que le mécanisme d'action de la 4-AP ne soit pas connu de façon précise, ses effets inotropes positifs peuvent s'avérer utiles lorsqu'il est nécessaire de renverser un bloc musculaire de type non dépolarisant chez des malades présentant une atteinte de la fonction myocardique de diverses étiologies. Cet agent serait contreindiqué chez des malades présentant une hypertension artérielle et/ou une atteinte coronarienne.