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CARDIOVASCULAR RESPONSES EVOKED FROM THE NICOTINE-SENSITIVE AREA ON THE VENTRAL SURFACE OF THE MEDULLA OBLONGATA IN THE CAT

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SUMMARY

1. Experiments were carried out in cats anaesthetized with chloralose, (a) to examine the effect on blood pressure, heart rate and respiratory frequency produced by topical application of leptazol, nicotine and sodium pentobarbitone to the ventral surface of the medulla at an area around the rootlets of the XII cranial nerve, and (b) to study the role of this area in some cardiovascular reflexes.

2. Leptazol applied uni- or bilaterally to this area produced hypotension, bradycardia and bradypnoea.

3. The area from which leptazol produced these effects was localized 3–6 mm lateral to the mid line and 5–9 mm caudal to the lower border of the trapezoid bodies.

4. When comparing the effects of leptazol and nicotine applied to this area it was found that in concentrations that produced similar falls in arterial blood pressure and heart rate leptazol produced a much stronger bradypnoea than nicotine.

5. The hypotension produced by leptazol was mainly due to inhibition of sympathetic vasomotor tone since it was little affected by section of the vagi and by atropine given intravenously.

6. Bilateral application of sodium pentobarbitone produced a small hypertension, tachycardia and pronounced tachypnoea. Unilateral application of sodium pentobarbitone had no effect by itself but inhibited the effects of leptazol applied to the same site.

7. Cardiovascular reflexes produced by sinus nerve stimulation, by increased sinus pressure or by injections of veratridine into a vein or into the left ventricle of the heart were potentiated by topical application of leptazol to the ventral surface and depressed by the topical application of sodium pentobarbitone.

8. The chemoreceptor reflex, produced by retrograde injections of lobeline into the lingual artery, was partially affected by topical application of sodium pentobarbitone: the evoked bradycardia was attenuated but the tachypnoea and hypertension were not affected.

9. These results suggest that this medullary area on the ventral surface of the medulla plays an important role in normal cardiovascular regulation.

INTRODUCTION

A number of studies in anaesthetized cats which have been reported in the past 15 years have established the existence, on the ventral surface of the medulla oblongata, of at least two distinct areas with different effects upon arterial blood pressure, respiration and vasopressin release (Feldberg & Guertzenstein, 1972; Guertzenstein, 1973; Guertzenstein & Silver, 1974; Bisset, Feldberg, Guertzenstein & Rocha e Silva, Jr, 1975; Feldberg & Guertzenstein, 1976; Feldberg, 1976). The more rostrally situated 'glycine-sensitive' area, in the vicinity of the trapezoid bodies, has been shown to be essential for the maintenance of normal arterial blood pressure (Guertzenstein & Silver, 1974). The more caudally situated 'nicotine-sensitive' area around the rootlets of the XII cranial nerve was revealed when it was shown that nicotine applied to it through paired Perspex rings induced increased vasopressin secretion and a lowered arterial blood pressure (Bisset, *et al.* 1975; Feldberg & Guertzenstein, 1976). The hypotensive effect of nicotine applied to a similar if not identical area was also observed by Dev & Loeschcke (1979) but they found, in addition, a rise in blood pressure when the nicotine was applied in weaker concentrations. This pressor effect had not been observed in the experiments of Feldberg & Guertzenstein (1976). This difference may be due to the fact that in the two sets of experiments different anaesthetics were used.

In the present experiments the functions of the nicotine-sensitive area were re-examined and its role in some cardiovascular reflexes was studied. For this purpose topical application of leptazol and sodium pentobarbitone were combined with sinus nerve stimulation, increased pressure in the carotid sinus, veratridine injections either intravenously or into the left ventricle of the heart, and chemoreceptor stimulation by lobeline. Leptazol was chosen since it is a non-specific excitant of neurones and sodium pentobarbitone because it has a depressant effect on neural activity.

In a few of these experiments the superior laryngeal nerve was stimulated electrically in order to evaluate possible interactions of respiratory responses with cardiovascular effects.

Some of the results have been communicated to the Physiological Society (Guertzenstein & Lopes, 1980).

METHODS

Cats of either sex weighing between 2·3 and 4·1 kg were anaesthetized with chloralose (50–60 mg kg^{-1} I.V.) after anaesthesia had been induced with ether to allow cannulation of the right femoral vein. Arterial blood pressure was recorded from a cannula inserted into the right femoral artery by means of a strain gauge transducer (Statham Instruments, model P 23Db) in turn connected to a galvanometric pen recorder (Beckman Instruments, model R611). Pulse pressure was recorded on one channel, attenuated mean pressure on a second channel and heart rate (derived by feeding the pressure signal into a cardiotachometer coupler, Beckman Instruments, model 9857B) on a third. The trachea was cannulated, and the respiratory movements were recorded by means of a differential transducer (Statham Instruments PM 15E) connected to the tracheal cannula and to a fourth channel. Artificial ventilation was applied only when prolonged respiratory arrest was caused by the application of drugs to the ventral surface of the medulla. In those experiments in which drugs were to be injected into the left heart ventricle a cannula was inserted into the left axillary artery and pushed into the heart chamber.

Topical application of drugs to the ventral surface of the medulla. The method of exposing the ventral

surface of the brain stem and of placing drugs on the ventral surface through paired Perspex rings was that previously described by Feldberg & Guertzenstein (1972); a diagram of the paired rings with their holder has been published (Guertzenstein, 1973). Drugs were applied in 10 μl volumes into each ring.

With leptazol a more precise localization was attempted. A single ring (3 mm diameter) was used which was attached to a micromanipulator so that it could be moved to various sites on the ventral surface. The site was afterwards determined by replacing the leptazol with Bromophenol Blue and observing its staining post mortem (see Feldberg & Guertzenstein, 1976).

Cooling of the vagal nerves. The trunks of the two vagal nerves were dissected in the neck and cooled to 4 °C by means of a water-circulating device. The temperature of the nerves was monitored by means of copper-constantan thermocouples.

Electrical stimulation of nerves. The sinus nerve and superior laryngeal nerve were dissected free from connective tissue on both sides but stimulation was applied unilaterally. The sinus nerve was stimulated in continuity, but the superior laryngeal nerve was cut peripherally prior to stimulation. Stimulation was applied by means of small bipolar platinum electrodes. Liquid paraffin was applied to the nerves at frequent intervals to prevent drying and to minimize the spread of the current during stimulation. Rectangular current pulses of 0·5 ms duration, 2–5 V amplitude and 20 Hz frequency were delivered by a Grass stimulator (S88) through a stimulus isolation unit (Grass SIU 5) (Lopes & Palmer, 1978).

Isolation of the carotid sinuses. Both carotid sinuses were partially isolated by tying all the arterial branches arising from the region of the carotid bifurcation, with the exception of the external and common carotid arteries. Cannulae were retrogradely introduced into each lingual artery and connected to a reservoir containing oxygenated Ringer solution beneath a cushion of compressed oxygen. The pressure within the sinuses was measured by a manometer coupled to the lingual artery cannulae. By placing clips on the external and common carotid arteries complete vascular isolation could be achieved. Between tests the clips were removed, restoring normal pulsatile blood flow.

Chemoreceptor stimulation. Injections of lobeline were made rectrogradelv through a cannula in the lingual artery with the external carotid temporarily occluded.

Materials. Ethyl ether (Rhodia), chloralose (E. Merck, Darmstadt), atropine methyl nitrate (Sigma), leptazol (Martindale), sodium pentobarbitone (Nembutal sodium powder, Abbott), lobeline (Boehringer, Ingelheim) and veratridine (Sigma) were used.

Substances applied topically to the ventral surface of the brain stem were freshly dissolved in 0·9% NaCl solution. The fresh leptazol and nicotine solutions were acidic; they were therefore neutralized to a pH of near 7·0 by the addition of small drops of 1 N-NaOH. Sodium pentobarbitone (30 mg ml^{-1}) solution was alkaline (pH 9·4). To test the effects of control solutions of the same alkalinity, small amounts of 0·2 N-KH_2PO_4 and 0·2 N-NaOH were added to 0·9% NaCl solution; these caused none of the effects of sodium pentobarbitone.

Statistical analysis. All statistical comparisons were performed by means of the paired *t* tests. In the Results, every reference to differences between values indicates a significant difference of $P < 0.01$.

RESULTS

The effects of leptazol application to the ventral surface

Bilateral application of leptazol through the paired Perspex rings placed on the ventral surface of the medulla 5–9 mm caudal to the trapezoid bodies (see diagram of Fig. 1) produced a fall in arterial blood pressure, bradycardia and respiratory depression, leading sometimes to respiratory arrest. These changes began 4–10 s after the leptazol application and, having reached plateau values, remained steady while the drug was kept in the rings. The return of blood pressure, heart rate and respiration to their control levels began as soon as the leptazol was washed out from the rings; full recovery took about 10–15 min.

In nine animals in which different concentrations of leptazol were applied the threshold concentration ranged between 10 and 20 mg ml^{-1}. With a concentration

of 50 mg ml^{-1}, mean arterial blood pressure (M.A.B.P.) decreased by $41{\cdot}6 \pm 8{\cdot}3$ mmHg (mean $\pm$ S.E. of the mean), heart rate (h.r.) by $60{\cdot}8 \pm 13{\cdot}5$ beats min^{-1} and respiratory frequency (r.f.) by $4{\cdot}4 \pm 0{\cdot}9$ breaths min^{-1}. With a leptazol concentration of 200 mg ml^{-1} the decreases were $78{\cdot}8 \pm 8{\cdot}2$ mmHg, $116{\cdot}8 \pm 14{\cdot}7$ beats min^{-1} and $8{\cdot}5 \pm 0{\cdot}6$ breaths min^{-1}. The control values for these animals were $139{\cdot}4 \pm 7{\cdot}5$ mmHg for M.A.B.P., $212 \pm 6{\cdot}6$ beats min^{-1} for h.r. and $12{\cdot}4 \pm 1{\cdot}2$ breaths min^{-1} for r.f.

Localization of the leptazol-sensitive area

Similar but weaker effects were produced with unilateral application of leptazol by means of a single ring (3 mm diameter). When the ring was placed on different regions of the ventral surface, to obtain a more precise localization of the sensitive area, leptazol (50 mg ml^{-1}) evoked its maximum effects when placed around the rootlets of the XIIth cranial nerve, circumscribing an area extending 5–9 mm from the caudal border of the trapezoid body and 3–6 mm from the mid line. This region which is shown in the diagram of Fig. 1 coincides with the one described for the hypotensive effects of nicotine in atropinized cats (Feldberg & Guertzenstein, 1976). When the ring was moved to more caudal positions, all three effects became weaker; with the rostral limit of the region covered by the ring more than 10 mm from the caudal border of the trapezoid bodies, the three effects were absent. On the other hand, with the ring placed in a more rostral position, i.e. within 5 mm from the caudal border of the trapezoid body, a dissociation of the three effects occurred inasmuch as the evoked hypotension became weaker and eventually turned into hypertension, although bradacardia and respiratory depression still occurred. Such dissociation was observed even when the most rostral edge of the ring was 2 mm rostral to the caudal border of the trapezoid bodies. No changes were obtained in blood pressure, heart rate or respiration with leptazol applied less than 3 mm from the mid line, irrespective of the position in rostro-caudal direction.

These findings show that the area from where bradycardia and bradypnoea were obtained was larger, in the rostral direction, than the one from where the fall in blood pressure was evoked by leptazol applications. The area is thus also larger than the nicotine-sensitive area; but it has to be remembered that the nicotine-sensitive area was mapped out for the hypotensive effect alone (Feldberg & Guertzenstein, 1976) since the experiments were done in atropinized cats and respiration was not recorded.

Comparison of the effects of leptazol and nicotine

In four cats in which the effects of bilateral application of leptazol and nicotine were compared it was found that doses which produced similar falls in blood pressure did not inhibit respiratory rate to the same extent. In the experiment of Fig. 1, for instance in which nicotine (2 mg ml^{-1}) and leptazol (50 mg ml^{-1}) produced a fall of 60 mmHg in M.A.B.P., the respiratory rate decreased by 3 min^{-1} with nicotine and by 12 min^{-1} with leptazol. A 4-fold increase of the concentration of these two substances did not significantly alter this disparity. At these concentrations both drugs produced a fall in blood pressure of similar magnitude but 8 mg ml^{-1} nicotine caused only a transient slowing of respiration for about 20 s, after which respiratory movements returned nearly to normal while 200 mg ml^{-1} leptazol produced intense respiratory slowing that lasted as long as the drug remained in the rings. Further,

in doses which produced similar falls in blood pressure the two drugs also caused similar reductions in heart rate. With nicotine (2 mg ml^{-1}) there was only slight, with leptazol (50 mg ml^{-1}) pronounced, sinus arrhythmia, but on application of higher concentrations of either of these drugs, sinus arrhythmia became less apparent or was

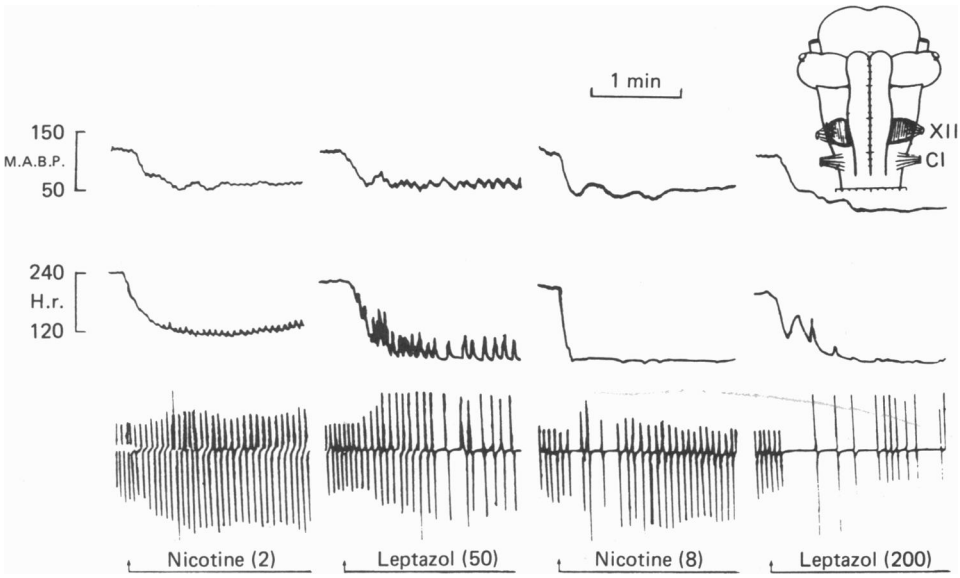

Fig. 1. Mean arterial blood pressure (M.A.B.P., mmHg), heart rate (h.r., beats min^{-1}) and respiratory changes recorded in a 2·8 kg cat. The horizontal lines indicate 2 min periods of bilateral application of nicotine and leptazol to the ventral surface of the medulla through Perspex rings placed in the position shown in the inset. Figures in parentheses indicate the concentration of the drug in mg ml^{-1}. Interval between applications, 50–60 min. XII: Hypoglossal nerve rootlets; CI: cervical I rootlets.

even absent. In these particular experiments leptazol (200 mg ml^{-1}) often caused respiratory arrest, but nicotine at 8 mg ml^{-1} did not do so. Nicotine did however produce respiratory arrest in one experiment in which it was applied in a concentration of 24 mg ml^{-1}.

Effects of cutting or cooling the vagi and of atropine

The fall in blood pressure produced by topical application of leptazol was mainly due to inhibition of sympathetic vasomotor tone, inasmuch as it was not substantially reduced by section or cooling the vagi or by the administration of intravenous atropine.

The traces in Fig. 2*A*, all of which were obtained from the same animal illustrate the effects of vagal section and atropine. In each record leptazol (200 mg ml^{-1}) was applied bilaterally to the nicotine-sensitive area. In Fig. 2*A* 1 blood pressure fell 90 mmHg and heart rate decreased from 230 to 130 beats min^{-1}; in *A* 2 after section of the vagi, blood pressure fell by 80 mmHg on renewed leptazol application, but now heart rate decreased only from 240 to 220 beats min^{-1}. The fall in blood pressure was not due to activation of sympathetic cholinergic vasodilator fibres to skeletal muscles

since as shown in Fig. 2*A*3 it was obtained also after intravenous atropine methyl nitrate (2 mg kg^{-1}). Both the fall in blood pressure and in heart rate were similar in traces 2 and 3 of Fig. 2*A*. To confirm that the fall in blood pressure was only partially due to the slowing of the heart provoked by an action of the vagi, the latter were temporarily blocked by cooling during the fall in blood pressure produced by the

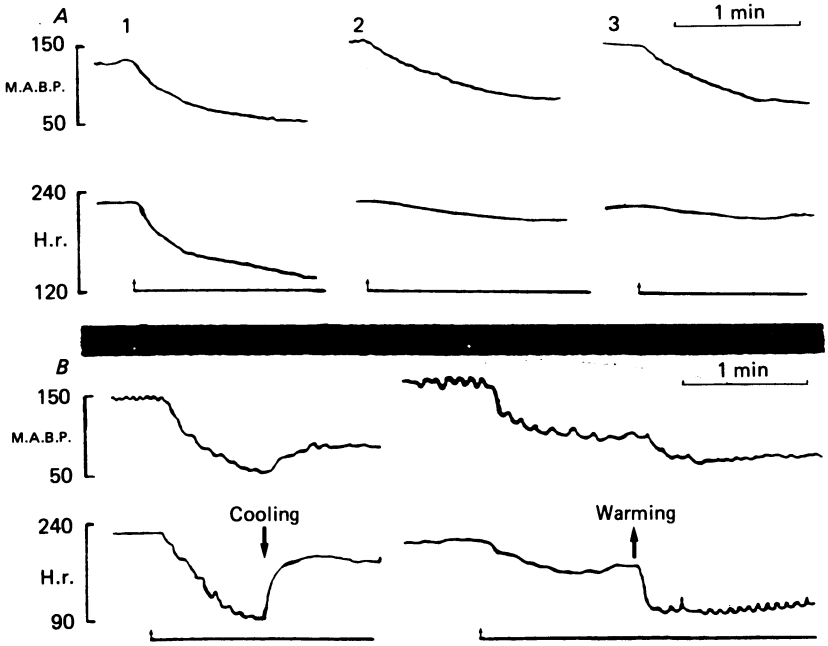

Fig. 2. Mean arterial blood pressure (M.A.B.P., mmHg) and heart rate (h.r., beats min^{-1}) recorded from a 2·8 kg cat (*A*) and a 3·5 kg cat (*B*). The horizontal lines indicate in *A*, 2 min periods and in *B*, 4 min periods of bilateral application of leptazol (200 mg ml^{-1}), to the area at the ventral surface shown in the inset of Fig. 1. Between *A*1 and *A*2 the vagi were cut, between *A*2 and *A*3 the cat was given atropine methyl nitrate (2 mg kg^{-1} I.V.). The arrows in *B* indicate cooling and warming of the vagi. The right hand records in *B* were obtained with the vagi cooled before the leptazol application. Interval between applications, 40–60 min.

topical application of leptazol. The result is shown in the traces of Fig. 2*B* which were obtained from another animal. Again leptazol (200 mg ml^{-1}) was applied to the nicotine-sensitive area. Blood pressure fell by 100 mmHg and heart rate diminished by 140 beats min^{-1}. When the vagi were then cooled heart rate rose by 120 beats min^{-1}, yet blood pressure rose by only 30 mmHg. Fifty minutes later this procedure was reversed. When leptazol was applied with the vagi cooled this led to a fall in blood pressure of 70 mmHg and a decrease in heart rate of 20 beats min^{-1}. When the vagi were then warmed with the leptazol still in the rings there was a steep fall in heart rate of 120 beats min^{-1}, yet blood pressure fell by only 20 mmHg. These effects were observed in two further experiments.

The effects of sodium pentobarbitone application to the ventral surface

Sodium pentobarbitone (30 mg ml^{-1}), applied unilaterally to the nicotine-sensitive area, did not produce any change in blood pressure, heart rate or respiration. However, applied bilaterally to this area it produced a pronounced tachypnoea, some

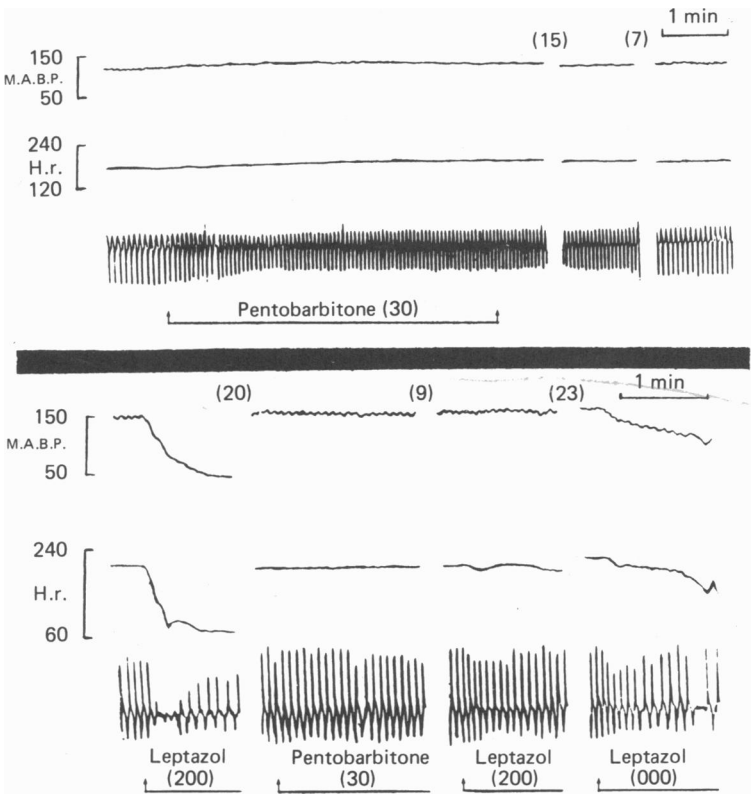

Fig. 3. Mean arterial blood pressure (M.A.B.P., mmHg), heart rate (h.r., beats min^{-1}) and respiratory movements recorded from a 2·5 kg cat (top records) and a 2·7 kg cat (bottom records). In the top records the horizontal line indicates a 4 min period of bilateral application of sodium pentobarbitone (30 mg ml^{-1}), in the bottom records the horizontal lines indicate 2 min periods of unilateral application of either leptazol (200 mg ml^{-1}) or sodium pentobarbitone (30 mg ml^{-1}) to the area shown in the inset of Fig. 1. Figures in parentheses on top of records indicate time interval between records (min).

increase in heart rate and a small but definite rise in blood pressure. This is illustrated by the traces in the upper part of Fig. 3, which were taken from an experiment in which the sodium pentobarbitone remained in the rings for 5 min. In less than 1 min after its application respiratory rate rose from 13 to 18 min^{-1}, heart rate from 180 to 198 beats min^{-1}, and blood pressure from 125 to 135 mmHg. None of these variables changed while the drug remained in the rings. When sodium pentobarbitone was then removed blood pressure returned to its control level within 15 min, but heart rate and respiration returned to control levels only after a further 7 min. In fact, whenever sodium pentobarbitone was applied bilaterally, the changes in heart rate

and respiratory rate lasted longer than the changes in blood pressure and sometimes blood pressure returned to control levels even before the pentobarbitone had been washed out of the rings. Such a recovery was never observed with respiration or heart rate. In six experiments the following increases were evoked by the topical application of sodium pentobarbitone (30 mg ml^{-1}): M.A.B.P. $13{\cdot}2 \pm 1{\cdot}1$ mmHg, h.r. $25{\cdot}7 \pm 4{\cdot}1$ beats min^{-1} and r.f. $8{\cdot}6 \pm 2{\cdot}3$ breaths min^{-1}. Control values for this group were: M.A.B.P. $156{\cdot}6 \pm 5{\cdot}6$ mmHg, h.r. $187.4 \pm 7{\cdot}8$ beats min^{-1} and r.f. $13{\cdot}3 \pm 0{\cdot}7$ breaths min^{-1}.

Even though sodium pentobarbitone applied unilaterally with the 3 mm diameter single ring to the nicotine-sensitive area did not affect blood pressure, heart rate or respiration, it inhibited the effect of leptazol subsequently applied unilaterally to the same site on the same side. This is illustrated in the lower records of Fig. 3 which show the effects of unilateral application of leptazol before and after unilateral application of sodium pentobarbitone. Five minutes after the 2 min application of sodium pentobarbitone, which by itself had been inactive, leptazol application no longer affected blood pressure, heart rate and respiration, but after 24 min its effects on blood pressure, heart rate and respiration began to return.

Effects of leptazol and sodium pentobarbitone application on cardiovascular and respiratory reflexes

The involvement of the nicotine-sensitive area in various cardiovascular reflexes was investigated by testing these reflexes during bilateral application of either leptazol or sodium pentobarbitone to this area. For these experiments leptazol, which mimics some of the effects produced by these reflexes, was applied bilaterally at a concentration of 10 mg ml^{-1} which is subthreshold for producing significant effects on blood pressure, heart rate or respiratory rate. Sodium pentobarbitone which abolishes the effects of leptazol was also applied bilaterally at a concentration of 30 mg min^{-1} which produces moderate cardiovascular effects and tachypnoea.

(*i*) *Sinus nerve and superior laryngeal nerve stimulation.* The bilateral application of leptazol at the subthreshold concentration of 10 mg ml^{-1} enhanced the cardiovascular effects evoked by electrical stimulation of the sinus nerve, which then produced a more powerful bradycardia and hypotension. This is illustrated in Fig. 4*A* which shows that the fall in blood pressure evoked by sinus nerve stimulation was enhanced from 10 to 35 mmHg and the bradycardia from 12 to 67 beats min^{-1}. This enhancement was reversible and the evoked responses returned control values after the leptazol had been washed out. In five similar experiments, sinus nerve stimulation produced the following decreases in blood pressure and heart rate: M.A.B.P. $17{\cdot}1 \pm 2{\cdot}8$ mmHg; h.r. $42{\cdot}5 \pm 11{\cdot}8$ beats min^{-1} before the leptazol application, and during its application the corresponding values were: M.A.B.P. $36{\cdot}4 \pm 5{\cdot}4$ mmHg; h.r. $96{\cdot}0 \pm 13{\cdot}9$ beats min^{-1}. These changes are significantly larger than the control changes.

Bilateral application of the subthreshold concentration of leptazol also enhanced the moderate decrease in respiratory rate produced by stimulation of the superior laryngeal nerve with a threshold current. This is illustrated in Fig. 4*B*. In five similar experiments this procedure resulted in a significant increase in respiratory pauses from $10{\cdot}5 \pm 0{\cdot}7$ to $16{\cdot}5 \pm 0{\cdot}6$ s.

The bilateral application of the sodium pentobarbitone to the nicotine-sensitive area had the opposite effect to that of leptazol. As shown in the experiment of Fig. 4*C* it virtually abolished the fall in blood pressure, heart rate and in respiration evoked by sinus nerve stimulation. In five similar experiments in which sinus nerve

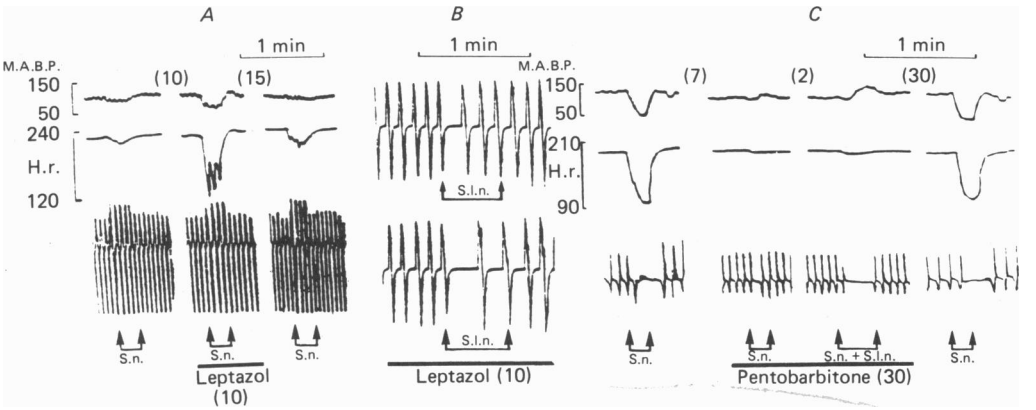

Fig. 4. Mean arterial blood pressure (M.A.B.P., mmHg), heart rate (h.r., beats min^{-1}) and respiratory movements recorded from a 3·2 kg cat (*A*) and a 4·0 kg cat (*C*), and respiratory movements from a 2·6 kg cat (*B*). Horizontal lines between arrows correspond to sinus (s.n.) and/or superior laryngeal nerve (s.l.n.) stimulation as indicated. Parameters of stimulation: 0·5 ms, 20 Hz, voltage varied between 2 and 3 V remaining constant in each experiment. Bottom horizontal lines indicate bilateral application of leptazol (10 mg ml^{-1}) or sodium pentobarbitone (30 mg ml^{-1}) to the area shown in the inset of Fig. 1. In (*A*) and (*C*) the figures in parentheses above records indicate time interval between records (min); in (*B*) the interval between upper and lower record is 10 min.

stimulation produced a fall of $50·0 \pm 6·1$ mmHg in M.A.B.P. and $98·4 \pm 17·8$ beats min^{-1}, a second period of sinus nerve stimulation, during the sodium pentobarbitone application, resulted in non-significant falls of M.A.B.P. ($7·0 \pm 2·5$ mmHg) and h.r. ($15·6 \pm 7·2$ beats min^{-1}). The slowing of respiratory rate was also almost abolished.

The bilateral application of sodium pentobarbitone did not affect the respiratory arrest produced by powerful stimulation of the superior laryngeal nerve but it had some inhibitory effect on the moderate bradypnoea produced by a weaker stimulation.

It was conceivable that the pronounced tachypnoea induced by the sodium pentobarbitone application could itself have suppressed the bradycardia evoked by sinus nerve stimulation (Lopes & Palmer, 1978). However this was not the case. When combining the sinus nerve stimulation during the sodium pentobarbitone application with a powerful stimulation of the superior laryngeal nerve there was respiratory arrest but no reduction in heart rate as illustrated in Fig. 4*C*.

(*ii*) *Increasing pressure in the isolated carotid sinuses.* The reflex fall in blood pressure, heart rate and respiratory frequency produced by increasing the pressure simultaneously in both isolated carotid sinuses was also potentiated by leptazol and inhibited by sodium pentobarbitone bilaterally applied to the nicotine-sensitive area. In five experiments in which the interaction between subthreshold leptazol (10 mg ml^{-1}) and increased sinus pressure was examined the fall in M.A.B.P. increased

from 18·0 ± 3·4 to 50·1 ± 8·2 mmHg and the fall in h.r. from 8·6 ± 1·2 to 26·8 ± 3·2 beats min^{-1}. The effect was significant with respect to both variables. The application of sodium pentobarbitone produced directly opposite effects, as illustrated in the experiment of Fig. 5*A*. The records show that before the sodium pentobarbitone application an increase of pressure in both carotid sinuses from 0 to 250 mmHg

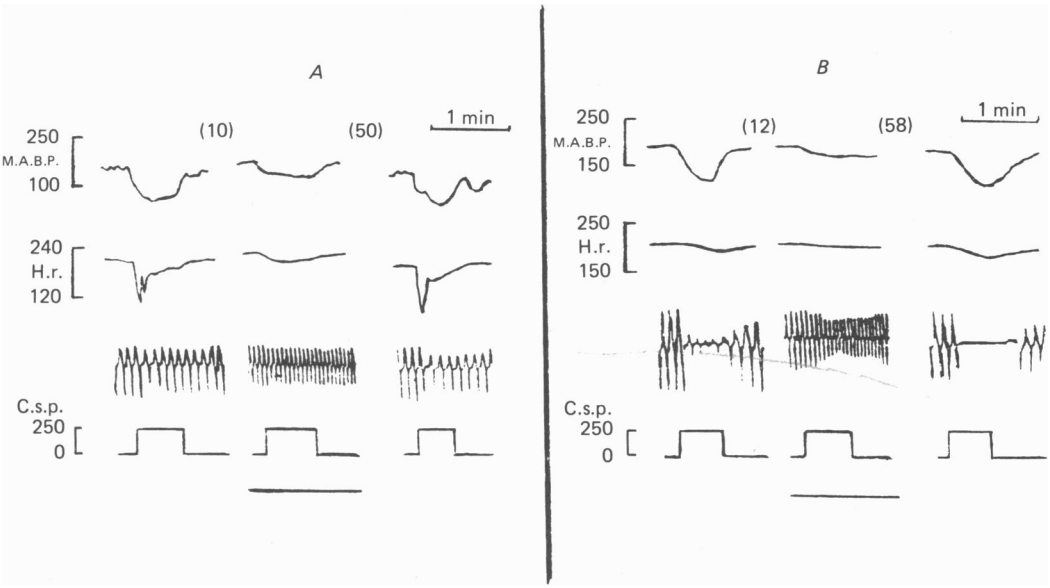

Fig. 5. Mean arterial blood pressure (M.A.B.P., mmHg), heart rate (h.r., beats min^{-1}) and respiratory movements recorded from a 3·5 kg cat (*A*) and a 3·1 kg cat (*B*) both of which had isolated carotid sinuses on each side. Variations in carotid sinus pressure (c.s.p., mmHg) are indicated by the lower trace. Horizontal lines indicate bilateral applications of sodium pentobarbitone (30 mg ml^{-1}) to the area shown in the inset of Fig. 1. Figures in parentheses on top of records indicate time interval between records (min). In experiment (*B*) the vagi had been sectioned previously.

produced a fall in M.A.B.P. of 70 mmHg, a fall in heart rate of 100 beats min^{-1} and some slowing of respiration. During the application the same rise in pressure in both sinuses produced a fall in M.A.B.P. of 20 mmHg, a fall in heart rate of 20 beats min^{-1} and the slowing of respiration did not occur. Fifty minutes after removal of the sodium pentobarbitone the evoked responses had returned to their control values. In two other similar experiments the same result was obtained.

This inhibition produced by sodium pentobarbitone application of the reflex fall in blood pressure was also observed in two experiments after vagotomy (Fig. 5*B*). The rise of pressure in the carotid sinuses resulted in a 70 mmHg fall of M.A.B.P, slight bradycardia and pronounced respiratory inhibition. During application of sodium pentobarbitone the same procedure evoked only a 15 mmHg fall in M.A.B.P., the slight bradycardia was abolished and the inhibitory effect on respiration was greatly reduced. Again the effects of sodium pentobarbitone were reversible.

(*iii*) *Intravenous and intracardiac veratridine injections.* Intravenous injections of veratridine (5–10 μg) induced reflex bradycardia, hypotension and bradypnoea.

These effects were potentiated by topical application of leptazol in subthreshold concentrations and virtually abolished by the topical application of sodium pentobarbitone.

In six experiments with leptazol its topical application increased the hypotensive effect of intravenous veratridine (10 μg) from 11·6±3·0 to 40·0±5·4 mmHg and

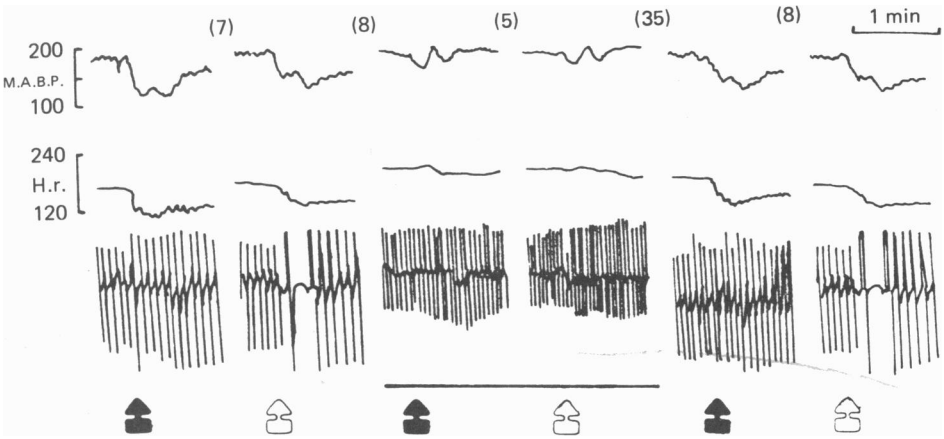

Fig. 6. Mean arterial blood pressure (M.A.B.P., mmHg), heart rate (h.r., beats min^{-1}) and respiratory movements recorded from a 2·9 kg cat. Arrows indicate injections of 10 μg veratridine into the left heart ventricle (closed arrows) and I.V. (open arrows). Horizontal line indicates bilateral application of sodium pentobarbitone (30 mg ml^{-1}) to the area shown in the inset of Fig. 1. Figures in parentheses above records indicate time interval between injections (min).

bradycardia from 30·0±3·6 to 83·5±8·2 beats min^{-1}. For both variables the effect of leptazol is significant. The bradypnoea was also more pronounced during the leptazol application.

In five experiments with sodium pentobarbitone its topical application decreased the hypotensive effect of intravenous veratridine (10 μg) from 66·0±6·9 to 17·0±3·7 mmHg and the bradycardia from 114·8±9·7 to 26·0±4·6 beats min^{-1}; the bradypnoea almost disappeared.

Injections of veratridine directly into the left ventricle of the heart also produced reflex hypotension and bradycardia but without changes in respiratory rate. In two such experiments the cardiovascular effects were abolished by topical application of sodium pentobarbitone.

The interaction of the sodium pentobarbitone application with the effects of intravenous and intracardiac veratridine injections is illustrated in Fig. 6 which shows that the effects were nearly abolished by the sodium pentobarbitone. A few minutes later after washing out the sodium pentobarbitone the effects of veratridine were again elicited.

(*iv*) *Stimulation of carotid chemoreceptors by lobeline injections.* Sodium pentobarbitone applied to the nicotine-sensitive area abolished the bradycardia, but not the hypertension and tachypnoea resulting from the chemoreceptor stimulation by lobeline. Injections of 10 μg lobeline into the lingual artery produced bradycardia,

hypertension and tachypnoea (Fig. 7) During bilateral application of sodium pentobarbitone, lobeline injections again produced hypertension and tachypnoea but no longer evoked bradycardia. This happened even when the lobeline was injected during the respiratory arrest produced by superior laryngeal nerve stimulation. The effect of sodium pentobarbitone disappeared after washing out the drug from the rings.

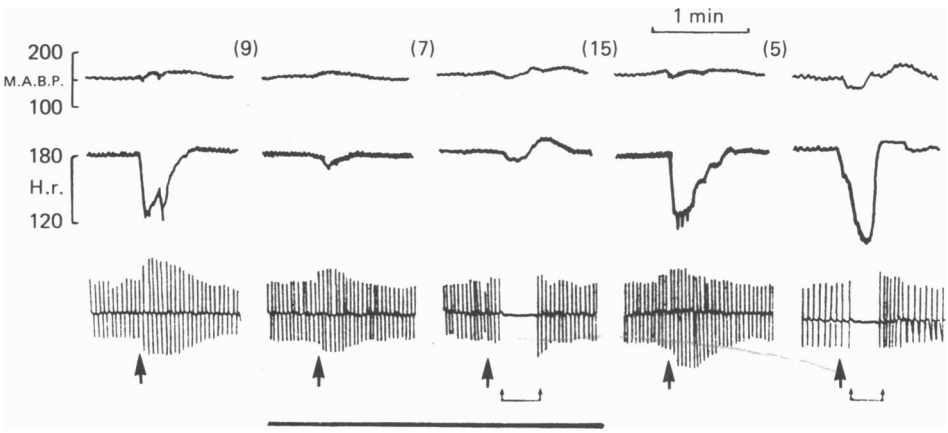

Fig. 7. Mean arterial blood pressure (M.A.B.P., mm Hg), heart rate, (h.r., beats min^{-1}) and respiratory movements recorded from a 3·2 kg cat. The large arrows indicate lobeline injections (10 μg) into the lingual artery. Horizontal lines limited by small arrows indicate periods of superior laryngeal nerve (s.l.n.) stimulation. Parameters of stimulation: 0·5 ms, 3V, 20 Hz. Bottom horizontal line indicates bilateral application of sodium pentobarbitone (30 mg ml^{-1}) to the area shown in the inset of Fig. 1. Figures in parentheses above records indicate time interval between records (min).

In four similar experiments stimulation of the chemoreceptors by lobeline increased M.A.B.P. by $14{\cdot}0 \pm 2{\cdot}3$ mmHg, r.f. by $4{\cdot}3 \pm 0{\cdot}8$ breaths min^{-1} and decreased h.r. by $75{\cdot}3 \pm 6{\cdot}2$ beats min^{-1}. The corresponding values during the sodium pentobarbitone application were: M.A.B.P., $16{\cdot}3 \pm 2{\cdot}7$ mmHg; r.f., $3{\cdot}4 \pm 0{\cdot}9$ breaths min^{-1} and h.r., $12{\cdot}3 \pm 3{\cdot}4$ beats min^{-1}. The difference in the values of M.A.B.P. and of r.f. under the two conditions was not significant, but the difference in the values of h.r. was.

In four experiments in which the lobeline injections were given during the maximum reduction in blood pressure, heart rate and respiratory frequency produced by bilateral application to the nicotine-sensitive area of leptazol (at the strong concentration of 200 mg ml^{-1}) they resulted in short-lasting increases of blood pressure ($17{\cdot}6 \pm 3{\cdot}1$ mmHg) and respiratory frequency ($5{\cdot}2 \pm 1{\cdot}1$ breaths min^{-1}).

DISCUSSION

The area from which hypotension and enhancement of a number of cardiovascular reflexes were obtained in the present experiments on topical application of leptazol and from which these reflexes were depressed on topical application of sodium pentobarbitone, is the same area from which hypotension has been produced

previously by application of nicotine. This area has been referred to as the nicotine-sensitive area. Leptazol was shown to have opposite effects on blood pressure when applied to the more rostrally situated glycine-sensitive area, as it produces a pressor effect from this area. Sodium pentobarbitone also acts differently on blood pressure when applied to the two areas, producing a strong depressor effect from the glycine and a weak pressor response from the nicotine-sensitive area (Feldberg & Guertzenstein, 1976).

The nicotine-sensitive area is approximately the same as the chemosensitive area L of Loeschcke and his co-workers (Trouth, Loeschcke & Berndt, 1973*a*). From this area they had obtained respiratory effects on electrical stimulation, and from an application of drugs or solutions of different pH (see Loeschcke, 1982). In some of these experiments blood pressure changes were observed as well; on electrical stimulation of this area however the effects on blood pressure consisted mainly of pressor responses (Trouth, Loeschcke & Berndt, 1973*b*). The difference between the effect of electrical stimulation and leptazol application could be explained if leptazol excites cell bodies or dendrites as discussed below, whereas electrical stimulation also excites nerve fibres which pass through this region. From experiments by Armendt, Czachurski, Dembowsky & Seller (1978) it is evident that neurones from the more rostrally situated glycine-sensitive area project to the thoracic intermedio-lateral column of the spinal cord where the preganglionic sympathetic fibres originate. If these fibres were to run beneath the nicotine-sensitive area close to the ventral surface they could well be excited from the stimulating electrode.

The idea that the action of both leptazol and sodium pentobarbitone is most likely to be at the level of nerve cells situated superficially, and not on axons of a pathway running near the surface in the area covered by the rings, is supported by the results of experiments in which the site of action of these two substances was examined in other regions of the C.N.S. For instance leptazol applied by micro-injection to the cerebral cortex produced its excitatory effects only when the injection was made into the grey and not into the white matter (Banerjee, Feldberg & Georgiev, 1970), and pentobarbitone applied to slices of olfactory cortex was found to depress synaptic transmission by interfering with the process involved in chemical transmission and not by blocking impulse conduction in the terminal branches of afferent nerves (Richards, 1982). Topically applied leptazol could excite synaptic sites over the perikaryon and/or on the superficially situated dendritic tree of cell bodies at some distance from the surface itself. For sodium pentobarbitone however, it would be difficult to envisage the possibility that inactivation of a superficial dendritic tree or perikaryon could lead to distant neural depression.

On the basis of previous experiments it is possible to conclude that if a drug is able to produce a strong effect by unilateral application to the sensitive area this is indicative of an excitatory action, whereas if a drug needs to be applied bilaterally to do so this is indicative of an inhibitory action. For instance, the inhibitory hypotensive action of glycine remains small when applied unilaterally to the glycine-sensitive area in whatever concentration it is applied, whereas on bilateral application it can produce a profound fall in blood pressure similar to that produced by bilateral destruction of these areas (Guertzenstein & Silver, 1974). Accordingly the fact that leptazol produces strong effects on blood pressure, heart rate and

respiration whether applied bilaterally or unilaterally suggests its action is excitatory, whereas the fact that sodium pentobarbitone produced its effects only on bilateral application suggests its action is inhibitory.

The excitatory action of leptazol and the inhibitory action of sodium pentobarbitone appear to be on the same neurones in the nicotine-sensitive area, since sodium pentobarbitone applied unilaterally to this area did not produce any effects on its own yet it inhibited the cardiovascular and respiratory effects of leptazol subsequently applied to the same site.

The topical application of leptazol must excite different groups of neurones at the ventral surface when producing its different effects, each effect apparently being the result of excitation of a different group of nerve cells. For when localizing the area of leptazol sensitivity by means of a single ring the area from which the cardiac and respiratory effects were obtained was found to be larger than the area from which blood pressure was affected and to extend more rostrally even beyond the nicotine-sensitive area. Further, when comparing the effects obtained with leptazol and with nicotine applied to the nicotine-sensitive area it was found that at concentrations which produced approximately equal circulatory effects leptazol produced a much stronger inhibition of respiration than nicotine. The first result differentiates between nerve cells at the ventral surface responsible for effects on blood pressure and nerve cells responsible for the cardiac and respiratory effects. The second result differentiates between nerve cells responsible for the respiratory effects and those responsible for the blood pressure and cardiac effects. Thus it seems that each of these three effects is brought about by excitation of a different group of nerve cells, and in the nicotine-sensitive area all three groups are present.

Further evidence for this differentiation was obtained from the experiments which dealt with the reflex activity evoked by veratridine injected into the left ventricle of the heart and by stimulation of the superior laryngeal nerve. Both of these reflex effects were abolished or reduced by the topical application of sodium pentobarbitone to the nicotine-sensitive area; they must therefore be dependent on neurones which originate in this area. The attenuation of these effects differentiates between the neurones projecting from these areas to the cardiovascular and those projecting to the respiratory system since the veratridine injections produced cardiovascular effects without affecting respiration, as first shown by Dawes (1947), and stimulation of the superior laryngeal nerve resulted in respiratory inhibition without producing cardiovascular effects. In corresponding experiments Lopes & Palmer (1978) obtained respiratory inhibition with bradycardia on stimulation of the superior laryngeal nerve. The fact that their cats were anaesthetized with chloralose plus urethane, whereas in the present experiments chloralose alone was used, may account for this difference.

In cats, Coote & Macleod (1974*a*, *b*) found fairly large groups of catecholamine-containing cells scattered along the ventro-lateral border of the medulla. Further they obtained sympatho-inhibitory responses when stimulating an 'area along the ventral surface of the medulla extending from the lateral nucleus to the mid line'. 'The catecholamine-containing cells were found beneath the lateral part of this area which would coincide with the nicotine-sensitive area'. The axons of these neurones were found to descend directly to the spinal cord. From these findings and those of

Fleetwood-Walker & Coote (1981) it would appear that the catecholamine-containing neurones which have their cell bodies beneath the nicotine-sensitive area (A_1 neurones) provide the main sources of output to the regions of the sympathetic preganglionic cell bodies in the spinal cord and are mainly concerned with regulating sympathetic activity, being part of a sympatho-inhibitory system and mediating the spinal component of baro-receptor inhibition.

The results of the present experiments are in agreement with these conclusions and suggest that additional important functions are mediated via the neurones originating in the region of the nicotine-sensitive area. From the results obtained with veratridine injections and stimulation of the superior laryngeal nerve it is evident that these neurones are involved in reflexes whose afferent pathways originate in the heart, the lungs or respiratory tract.

By contrast only part of the chemoreceptor reflex produced by intracarotid lobeline could be dependent on neurones originating in the nicotine-sensitive area, because neither the hypertension nor the tachypnoea but only the bradycardia evoked by the lobeline injection was inhibited by the application of sodium pentobarbitone to this area. This inhibition of the lobeline-induced bradycardia is a genuine inhibitory effect of the sodium pentobarbitone on this reflex and not the indirect result of the tachypnoea produced by the sodium pentobarbitone application, tachypnoea itself being known to reduce vagal activity to the heart (Palmer & Lopes 1978). This possibility was excluded by showing that the pentobarbitone application continued to prevent the lobeline bradycardia when combined with strong stimulation of the superior laryngeal nerve which itself caused respiratory arrest.

Further evidence that at least the hypertension evoked by the lobeline is not mediated via neurones originating in the nicotine-sensitive area, was the finding that this hypertension was also obtained during application to this area of a concentration of leptazol which strongly inhibited sympathetic activity.

Although the application of sodium pentobarbitone to the nicotine-sensitive area nearly abolished an evoked baroreceptor reflex, it only produced a minor rise in the base line level of blood pressure and heart rate. This apparently paradoxical result may be compared with the observations of Miura & Reis (1972), who showed that although destruction of the solitary tract nuclei in anaesthetized cats abolished the cardiovascular components of baro- and chemoreceptor reflexes, it did not raise the resting level of blood pressure or heart rate. Yet an increase of 50 % in blood pressure occurred as soon as the animal recovered from anaesthesia (Nathan & Reis, 1977). In the present experiments, the anaesthesia may have been responsible for the fact that the base line level of blood pressure and heart rate was scarcely affected by the sodium pentobarbitone application. It is conceivable that destruction of this area performed in anaesthetized cats would also scarcely affect the existing level of blood pressure and heart rate but that blood pressure and heart rate would increase greatly as soon as the cats came out of anaesthesia. Such a result would prove that this area plays a key role in cardiovascular regulation in the conscious as well as in the anaesthetized state.

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