THE EFFECT OF N, N-DI-ISOPROPYL-N'-ISOAMYL-N'-DIETHYLAMINO ETHYLUREA (P-286) ON AORTIC AND CAROTID CHEMORECEPTORS

A THESIS

By

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INTRODUCTION

Intravenous Acetylcholine

The intravenous administration of small doses of acetylcholine in anesthetized cats produces a fall in arterial blood pressure. This action, termed "muscarinic" is mediated through peripheral cholinergic receptors and can be inhibited by an appropriate dose of atropine (8). Intravenous administration of larger doses of acetylcholine in the presence of atropine usually produces a rise in arterial blood pressure. The classical explanation for this effect, termed "nicotinic" by Dale (8) is that acetylcholine stimulates sympathetic ganglia and the adrenal medulla to release effective concentrations of catecholamines (8). The cardiovascular manifestation of the nicotinic action of acetylcholine may be abolished or reversed by classical ganglionic blocking agents (18).

P-286

More recently, studies of the pharmacologic properties of an alkyl substituted urea, N, N-di-isopropyl-N'-isoamyl-N'-diethylamino ethylurea (P-286) have yielded sufficient information to refute the classical interpretation for the nicotinic pressor response from acetylcholine in intact anesthetized animals. Some of the basis for such a conclusion is provided by the pharmacological properties of this compound that differ from those of classical ganglionic blocking agents.

Compound P-286 when given intravenously in doses adequate to reverse the pressor response to acetylcholine for a period of hours, itself produced a depressor response lasting for a few minutes (12). Conversely, hexamethonium, in doses that reverse the pressor response to acetylcholine in atropinized animals causes a long-lasting hypotension (12). Recovery of the nicotinic response to acetylcholine began a few minutes after the hexamethonium injection although the hypotension continued (12).

Contractions of the nictitating membrane resulting from preganglionic superior cervical nerve stimulation in the dog were unaltered by treatment with P-286, although these contractions were readily and significantly reduced by hexamethonium (12). Repetition of this work in the cat with measurement of nerve surface electrical activity demonstrated that P-286 had no effect on the "complex" postganglionic action potential arising from electric stimulation of the preganglionic superior cervical trunk. Chlorisondamine, another competitive ganglionic blocking agent, and hexamethonium abolished such postganglionic nerve activity (4). When recordings were made from a postganglionic sympathetic nerve possessing spontaneous activity, compound P-286 effectively suppressed that activity induced by acetylcholine without altering spontaneous

discharge (13). Chlorisondamine blocked both spontaneous activity and that induced by acetylcholine in the postganglionic nerve (13).

Such a dichotomy of action by P-286 suggested to these investigators that the activity induced by acetylcholine in the postganglionic sympathetic nerves did not originate in the ganglion. Recordings of preganglionic activity resulting from a nicotinic dose of acetylcholine confirmed this suspicion. Acetylcholine stimulated preganglionic nerve electrical activity which was reduced by P-286 (13). Consideration of the possible central nervous system origin of acetylcholine-induced sympathetic activity directed attention to the respiratory stimulation that occurs almost simultaneously with the blood pressure effect (13). Additional experiments demonstrated that the respiratory stimulation produced by nicotinic doses of acetylcholine was inhibited by P-286 (13).

A large number of nicotinic stimulants apart from acetylcholine have been found by Heymans et al. (15), to excite carotid and aortic chemoreceptors. Moe et al. (18), by abolishing respiratory stimulation induced by acetylcholine and nicotine with the ganglionic blocking agent tetraethylammonium, confirmed the nicotinic nature of the drug receptor at these sensing sites. Douglas (11) extended these findings to a more potent ganglioplegic agent, hexamethonium, and claimed in addition that the response to cyanide and anoxia was

enhanced. The enhancement of the response to sodium cyanide appears to be typical of classical competitive ganglionic blocking agents and affords another pharmacological contrast with P-286, since this compound does not possess such a property (13).

Increases in blood pressure associated with nicotine are blocked by ganglionic blocking agents. However, the pressor response to acetylcholine is reversed by these blocking agents. This effect was well demonstrated by Atanackovic and Dalgaard-Mikkelsen in their experiments on chemoreceptors and acetylcholine hypertension (2), in which chemoreceptor denervation reversed the pressor response to small doses of acetylcholine given in the presence of cholinesterase inhibitors. With larger doses of acetylcholine a secondary pressor component was observed which was dependent on the control blood pressure and abolished by adrenalectomy. These data suggested that the pressor response to acetylcholine (as reversed by P-286) was a product of chemoreceptor excitation and direct stimulation of the adrenal medulla.

Objective

Although the effect of P-286 on the adrenal medulla has been documented and absence of a ganglionic effect clearly demonstrated, the suppression of chemoreceptor activity by this compound has never been directly demonstrated. It is the purpose of this work,

therefore, to evaluate directly the effect of P-286 on chemoreceptor activity by recording afferent nerve activity from aortic and sinus nerves innervating sinus and aortic chemo- and baroreceptors following certain physiological and pharmacological stimuli.

Carotid and Aortic Chemoreceptors: History and Anatomy

The carotid body has been known to anatomists since 1793 and was thought by many to be glandular in nature (19). Innervation of the carotid body by the ninth and tenth cranial nerves had been noted in 1862 by Lushka, although stress was subsequently placed on its sympathetic innervation and on the presence of chromaffin tissue. De Castro in 1926 in a detailed histological study of the carotid body and nerve supply, emphasized its afferent innervation and suggested a possible chemoreceptor role for this tissue (5).

In the cat the carotid body is a small nodule 1-2 mm in diameter which weighs approximately 2 mg and is located on the occipital artery or the occipito-ascending pharyngeal trunk (1). Branches of the artery supplying the carotid body may supply the adjacent carotid sinus since they are closely associated anatomically. The venous drainage of the carotid body is quite variable, but it usually enters the internal jugular vein, which also receives blood from the superior cervical sympathetic ganglion and the nodose ganglion. The

blood flow per gram of tissue is the largest in the body (20 ml/gm/min) although the actual flow is very small (0.04 ml/min) (9).

In the carotid body of the cat are found several glomeruli separated by connective tissue. Histologically, two types of large glomerular cells have been distinguished on the basis of staining characteristics with hematoxylin and eosin and silver. The innervation of the glomerular cells is still a controversy. Current evidence indicates that the nerve endings do not enter the glomerular cells but are in close apposition (5).

In 1927, J.F. and C. Heymans, by means of perfusion experiments in which the aortic arch region was isolated, first demonstrated the presence of chemoreceptors in the region of the aortic arch. Three years later C. Heymans and co-workers described a similar function for the carotid body. Since that time the respiratory and cardiovascular ramifications of chemoreceptor function have been explored by a multitude of investigators (15, 19).

METHODS

Surgical Preparation

Adult cats of either sex, weighing from 1.5 kg to 3.5 kg were anesthetized with pentobarbital sodium (30 mg/kg I.V.) and maintained with small supplemental doses (5 mg/kg). A femoral vein was cannulated for drug injections and the opposite femoral artery was cannulated for blood pressure measurement. A ventral, cervical midline incision was made and the trachea with its associated muscles and the esophagus were removed to obtain an optimum field for visualizing the ninth and tenth cranial nerves. A cannula was inserted into the trachea immediately rostral to its entrance into the thoracic cavity. Using a dissecting microscope, the sinus nerve was carefully separated from the surrounding tissue and stripped of its connective tissue sheath with care taken not to injure the venous drainage of the sinus area. In some experiments the vagosympathetic trunk was removed from the carotid sheath. Three bundles of vagosympathetic fibers could be identified; the vagus, the cervical sympathetic, and the aortic nerves. The aortic nerve was verified by the oscilloscopic display of the rhythmic baroreceptor action potentials synchronized with the rise of each vascular pressure pulse by means of recording electrodes.

Procedures

Bipolar platinum electrodes (0.75 mm diameter) were placed under those nerves from which activity was to be measured. The surgical field was then flooded with mineral oil sufficient to cover the nerves and recording electrodes. The nerve potentials were amplified with a Grass dual 9AC preamplifier in series with a Tektronix 2A61 differential amplifier and displayed on a dual beam Tektronix oscilloscope. The sweep rate was 5 centimeters per second. Using a Grass kymographic camera each consecutive sweep of the electron beam was photographed and representative examples are presented in the pertinent figures. Chemoreceptor discharge was quantitated by counting the number of frames in which increased nervous activity could be observed.

Arterial blood pressure was measured with a Statham P-23 arterial pressure transducer and recorded on a Grass polygraph. Endotracheal pressure was used as a measure of respiratory frequency and depth. This was accomplished by wedging the end of a polyethylene tube into a small hole exposed in the side arm of the tracheal cannula. The other end of the tube was connected to a Statham P-23B venous pressure transducer. Changes in pressure caused by the animal's breathing were amplified and the deflections recorded on the polygraph.

To induce hypercarbia, animals were ventilated with a mixture of 90 per cent carbon dioxide and 10 per cent oxygen for a period of 30 or 60 seconds. To induce hypoxia, animals were given 100 per cent nitrogen to breath for one minute. To administer the above gases at atmospheric pressure a plastic bag partially filled with the appropriate gas was connected by means of Tygon tubing to the tracheal cannula. A low resistance non-rebreathing valve was placed between the trachea and the gas reservoir to provide minimum dead space, and inhalation of a constant gas mixture.

A total of sixteen experiments were completed. Two experiments recorded sinus nerve activity alone. In eight experiments arterial blood pressure was displayed on the oscilloscope together with sinus nerve activity. In two experiments both sinus and aortic nerve activity were measured concomitantly. In four experiments in which preganglionic cervical sympathetic activity was measured, two included aortic nerve activity, the other two sinus nerve activity.

Drugs used were acetylcholine chloride, sodium cyanide, atropine sulphate, dimethylphenylpiperazinium iodide, heparin sodium and P-286. Doses are expressed in terms of the salt. Duplicate doses of acetylcholine, sodium cyanide and DMPP were given before and after P-286. Injections were given intravenously

through a polyethylene catheter in the left femoral vein and washed in with one ml of normal saline. All animals were treated with 1 mg/kg of atropine and received challenging doses of acetylcholine and sodium cyanide. DMPP was used in those experiments (four) in which sympathetic activity was recorded. Hypercarbia and hypoxia were induced with carbon dioxide and nitrogen respectively in those experiments (six) in which nerve activity from two nerves was displayed.

Nitrogen and the carbon dioxide-oxygen mixture were obtained from Ohio Chemical and Surgical Equipment Company, Cleveland, Ohio. Statistical analysis was done using Student's "t" test for paired data (17).

RESULTS

Pharmacological Stimulation

The compound under study, P-286, was given in successive doses of 2 mg/kg every five minutes for a total of 6 mg/kg. This manner of administration was used to minimize the hypotension and negative chronotropic response and maximize the selective autonomic blockade. Although the hypotensive effect to each injection was precipitous it lasted only a few minutes, after which the pressure gradually returned almost to control level. The arterial pressure was slightly but significantly lower than before P-286, although not the basal pressure resulting from the use of typical ganglionic blocking agents (Table 1). A similar pattern may be noted in regard to the depression of cardiac rate by P-286 (Table 1).

Acetylcholine. Chemoreceptor activity, recorded as action potentials from the aortic and carotid sinus nerves, was stimulated by acetylcholine administered intravenously in amounts as small as 1 ug/kg. The duration of nerve activity from acetylcholine increased progressively with increases in dose. However, 1 mg/kg was the dose used most frequently. This dose produced changes in blood pressure, respiration and nervous activity of an intensity, duration and reproducibility adequate to allow a statistical evaluation of the blocking potency of the antagonist, P-286.

Figure 1 presents a typical example of the activity stimulated by acetylcholine in the aortic and carotid sinus nerves and its reduction by 6 mg/kg of P-286. The administration of an additional 6 mg/kg of this compound produced a further decrement in the response to acetylcholine, as can be noted from the experiment displayed in Figure 2. When this step-wise suppression was evaluated according to the criterion mentioned, each was found to be statistically significant (Table 2).

In three experiments activity in sympathetic efferent nerves was recorded concomitantly with that in one of the afferent nerve trunks. Figure 3 presents observations of the suppressant effect of P-286 on activity induced by acetylcholine in the preganglionic superior cervical aortic nerve. It is apparent that increased aortic nerve activity slightly precedes the elevated activity in the superior cervical nerve and persists for a longer time. Such data support the concept of reflexly induced sympathetic activity arising from a chemoreceptor source. It also may indicate that a minimum of activity in the chemoreceptor nerves is necessary to stimulate and maintain this reflex.

Despite the relatively consistent blocking effect of P-286 on chemoreceptor activity, the alteration of the induced respiratory pattern was quite variable. Figures 4 and 5 show the changes in

respiration and blood pressure attending the acetylcholine responses presented in Figures 1 and 3 respectively. These results indicate that the respiratory response is not a simple matter of chemoreceptor stimulation. Respiratory stimulation from acetylcholine was reduced unequivocally by P-286 in certain experiments (Figure 4), and not in others (Figure 5). The relationship of synchronous changes in blood pressure and depth of anesthesia to this respiratory response will be discussed later.

Regardless of the control response, treatment with P-286 was followed consistently by a transient hypotension after acetylcholine administration. This is aptly demonstrated in Figure 4 and 5 in which control responses consisted of either phasic changes in blood pressure or pressor activity composed of single or dual components.

Dimethylphenylpiperazinium. Studies of the suppressant effect of P-286 on pharmacological stimulation of peripheral chemoreceptors was extended to include the "ganglionic stimulant", DMPP, known to possess such activity. Since atropine had no observable effect on the responses to DMPP this drug might be considered a selective sympathetic nervous system stimulant. P-286 was as effective in reducing the stimulation of chemoreceptors caused by DMPP as it was in the case of acetylcholine (Figure 6). Furthermore, the

effect on respiration was dependent on several related variables already mentioned and as such will be discussed later. The respiratory recordings presented in Figure 7 are companion responses to the nerve activity in the preceding figure and display the inability of P-286 to block respiratory stimulation from DMPP. Conversely, alterations in sympathetic activity and blood pressure were affected in a parallel and well correlated manner.

Sodium Cyanide. The chemoreceptors of the carotid body appeared to be relatively more susceptible to stimulation by cyanide than were the aortic bodies. Although the amplification of the signal from the aortic nerve was less than that necessary with the sinus nerve, the disproportionate increase is observable from the recordings presented in Figure 8.

Increases in sympathetic activity produced by cyanide usually were quite variable. When distinct changes occurred, these were quantitatively parallel to aortic nerve activity. Such possible selectivity by cyanide for sinus afferent receptors could be the chief determinant of the cardiovascular response. The blood pressure recordings presented in Figures 9 and 10 justify the impression that increased sympathetic activity is not a prominent feature of cyanide action. Compound P-286 in doses of 6 and 12 mg/kg had no significant effect on the cardiovascular, respiratory or nervous activity produced by cyanide (Figure 11).

Physiological Stimulation

Hypercapnia. Very large concentrations of carbon dioxide were necessary to give a short onset and well defined stimulatory effect on peripheral chemoreceptors. In these experiments a 90 per cent concentration of this gas with oxygen provided a clear-cut stimulus. P-286 was without effect on the nervous and respiratory pattern as well as the hypotension resulting from the hypercarbic challenge. However, the secondary pressor response was abolished (Figure 12). Presumably the initial vasodepression was a product of the direct action of carbon dioxide on the peripheral vasculature and the subsequent rise in blood pressure was due to adrenal medullary discharge.

Hypoxia. As with the hypercapnic stimulus it was expedient to use a short-term exaggerated challenge, 100 per cent nitrogen inspired over a 60 second period. Recordings made from the aortic and carotid sinus nerves indicated that P-286 had no effect on the increased chemoreceptor activity so produced. Furthermore, P-286 fails to affect the respiratory changes and minor alterations in arterial pressure resulting from this limited period of hypoxia (Figure 13).

DISCUSSION

Although numerous factors control breathing, the final common pathways of respiration are the lower motor neurons which innervate the diaphragm and intercostal muscles. Rhythmic discharge of medullary respiratory center constitutes the minimal neural requirement necessary for rhythmic respiration. This basic rhythmicity is further refined or modified by the apneustic center which facilitates inspiration in the absence of inhibitory impulses. Impulses stimulating this area increase the depth of respiration. The pneumotoxic center residing in the rostral portion of the pons modulates the tonic activity of the apneustic center and thereby causes an increased rate and decreased depth of respiration.

Although integration at the level of the spinal cord undoubtedly occurs, the respiratory centers in the brain stem provide the mechanism for coordinating respiratory movements relative to rate and amplitude. Neural components from higher centers and a myriad of peripheral afferent systems impinge on these controlling centers. Stimulation of any dorsal root nerve will bring about reflex changes in respiration. Nerve elements giving origin to voluntary respiration are probably in the motor area of the cerebral cortex (20).

Despite the many centers and varied reflexes affecting respiration, the most important and undoubtedly the primary role is

played by chemical regulation and is indicated by the stability of partial pressure of carbon dioxide (pCO_2) of the circulating blood despite wide variation in carbon dioxide production. The modern concept of chemical regulation of respiration states that the medullary respiratory center is directly depressed by increased carbon dioxide or decreased oxygen (5). However, increased carbon dioxide or hydrogen ion concentration stimulates respiration by activation of central chemoreceptors possibly located in the lateral recesses of the fourth ventricle (7). These sensing neurons monitor the pH of the interstitium adjacent to the cerebrospinal fluid. The pH of the cerebrospinal fluid is a sensitive indicator of blood pCO_2 because it lacks the buffering capacity of whole blood and is relatively impermeable to bicarbonate ion. Therefore, alterations in pH will occur in proportion to pCO_2.

Although the influence of the carotid and aortic chemoreceptors in "normal" respiration is still somewhat controversial, their role in stimulating respiration in hypoxic states is well established. The carotid and aortic bodies are stimulated by hypoxia and to a lesser extent by carbon dioxide and a decreased pH of the blood. However, these last two increase the sensitivity of chemoreceptors to hypoxia.

P-286 consistently depressed the responses of the aortic and carotid chemoreceptors to intravenous acetylcholine and dimethylphenylpiperazinium. However, the respiratory response to these two agonists as well as the blocking effect of P-286 as determined from this parameter were quite inconsistent (Fig. 2, 4, 5). In general the respiratory response to any drug used, demonstrated little consistency even with identical doses in the same animal. Such variability was unquestionably a product of anesthetic depth, a factor difficult to control. Considering the complex pattern preceding the final emergence of activity from the lower motor neurons to activate the respiratory muscles, it would be inappropriate to evaluate the potency and effectiveness of any drug on peripheral chemoreceptors by merely observing respiratory movement unless extraordinary precautions were taken to maintain a constant level of anesthesia.

Barbiturates depress the central respiratory center and the central respiratory chemoreceptor to a greater extent than peripheral chemoreceptors (7). The depressant action of barbiturates, therefore, decreases the respiratory response to carbon dioxide more than that to hypoxia, since the stimulation from carbon dioxide is essentially central. The variable response to carbon dioxide in these experiments was indeed a measure of the fluctuation in

anesthetic depth. One of the few exceptions to this variable response to carbon dioxide may be seen in figure 12 where the similarity in response was undoubtedly a product of similarity in anesthetic level.

It was also apparent that the respiratory and cardiovascular responses induced by acetylcholine cannot be limited to their effect on chemoreceptors. Acetylcholine directly stimulates other affectors such as gustatory afferents, baroreceptors, chemosensitive afferents in the lung and vascular system (3). The initial inhibition of respiration sometimes seen with acetylcholine could be explained by either a stimulation of baroreceptors or the pulmonary chemoreflex which produces apnea followed by an increased rate of respiration. The high concentration of carbon dioxide used also initially inhibited respiration. This could be due to a chemical irritation of the lower respiratory tract.

The blood pressure alteration with the sodium cyanide dose shown in Figure 9, is in a direction opposite to that displayed in Figure 10. Such variability in response could well be related to a balance between sympathetic activity and respiratory response both reflexly induced by chemoreceptor stimulation. Although an explanation of this phenomenon is impossible from the present data, recent critical investigations separating aortic body from carotid body function are pertinent to the problem and bear discussion (6, 16).

By interposing a coil of polyethylene tubing in the common carotid arteries of the dog and examining the effects of agents in this experimental preparation, Comroe (6) demonstrated that hyperpnea was predominantly due to carotid body stimulation. This investigator also found that aortic body stimulation resulted in a response typical of sympathetic stimulation, whereas carotid body stimulation resulted in a vagotonic (parasympathomimetic) type of response. The greater influence of the carotid bodies in producing hyperpnea was confirmed by Daly (10) who separately perfused the peripheral chemoreceptor areas by cross circulation from donor animals. However, this author stated that the primary vascular response from both sets of chemoreceptors, is vasoconstriction, and that a reduction in systemic vascular resistance from carotid body stimulation is due to overriding secondary respiratory mechanisms. In the results presented herein (Figure 8) sodium cyanide produced a greater response in the sinus nerve than in the aortic nerve, and would be compatible with the observations of the authors mentioned above. It would follow, then, that an increase or decrease in blood pressure following sodium cyanide would be dependent on the respiratory response which in turn would be related to anesthetic depth. Again, referring to Figures 9 and 10 and considering all such data gathered in this study, no such relationship could be discerned.

Observations of the recording film either with or without magnification allowed an evaluation of nerve activity sufficient to discern unequivocal drug effects. Attempts to quantitate this nerve activity in terms of number of fibers involved or single fiber frequency by teasing out and recording from just a few fibers were not successful. Although surgical separation of baroreceptor and chemoreceptor fibers was not accomplished, their activities were readily distinguishable. Firstly, baroreceptor activity was synchronous with the pulse. Secondly, baroreceptor activity changed appropriately with the change in blood pressure. Thirdly, the chemoreceptor responses were appropriate to the stimulus while the blood pressure and the related baroreceptor responses were inconsistent and variable. Finally, the chemoreceptor response elicited by the injected drugs preceded any respiratory or cardiovascular effects, indicating that the initial response is due to a direct chemoreceptor stimulation.

In addition to the baro- and chemoreceptor afferent fibers present in the nerves used for recording, the possibility of efferent cranial or sympathetic nerve contamination must also be considered. In a number of experiments the sympathetic nerve entering the carotid sinus area and/or the glossopharyngeal nerve was sectioned without any apparent change in electrical activity.

It was hoped at the beginning of this work that compound P-286 would be a tool capable of inhibiting both pharmacological and physiological stimulation of chemoreceptors. This certainly was not the case and the clarification of the dichotomy between these two types of stimuli must await further investigation.

SUMMARY AND CONCLUSIONS

By measuring the electrical activity from the sinus and aortic nerve it has been shown that P-286 blocks the nicotinic stimulation of chemoreceptors by acetylcholine and DMPP.

These data are consistent with the role of chemoreceptors in the nicotinic response to acetylcholine although it does not indicate what other afferent sites may play a role in the respiratory and cardiovascular response to acetylcholine.

TABLE 1

The Effect of P-286 on Arterial Blood Pressure, Heart Rate and Respiratory Rate in the Anesthetized cat (a)

	Systolic B. P. (mm Hg)	Diastolic B. P. (mm Hg)	Heart Rate (beats/ min)	Resp. (breaths/ min)
Control	155.3(±28.4) (16)	110.6(±23.0) (16)	150.4(±27.3) (16)	12.8(±5.8) (10)
Response after P-286 6 mg/kg I. V.	135.3(±28.6)** (16)	90.2(±26.6)** (16)	120.2(±18.8)** (16)	12.5(±6.8) (10)
Response after P-286 12 mg/kg I. V.	127.0(±21.7)* (5)	81.0(±23.3) (5)	91.5(±6.4)*** (4)	--

(a) Values given are means ± the standard deviation with the number of experiments in parentheses.

* $P < .05$ as compared to 6 mg/kg I. V. of P-286.
** $P < .001$ as compared to control.
*** $P < .001$ as compared to 6 mg/kg I. V. of P-286.

TABLE 2

Duration of Chemoreceptor Activity in Response to Acetylcholine and Sodium Cyanide after P-286 6 mg/kg and 12 mg/kg I. V.
(duration in seconds)

Expt.	Control	after P-286 6 mg	after P-286 12 mg
1	28	10	6
2	12	6	2
3	18	4	3
4	16	4	2
5	8	4	1
mean S.D.	16.7(±9)	5.7(±3)*	2.8(±6)**
Sodium Cyanide 50 ml/kg			
1	10	12	-
2	7	10	12
3	18	22	16
4	14	16	14
5	13	15	17
mean S.D.	12.3(±3.8)	15.1(±3.8)	15.0(±2.8)

*These values are significantly different from control values ($P < .05$).
**These values are significantly different from values after P-286 6 mg/kg ($P < .05$).

Figure 1 Electrical activity in the sinus and aortic nerves during the response to acetylcholine 1 mg/kg I.V. before and after P-286.

Each frame represents a consecutive two-second sweep of the electron beam during the response to acetylcholine. The increase in sinus nerve activity starts near the end of the first frame. The increase in aortic nerve activity is nearly obscured by the larger baroreceptor activity. In the bottom row the initial increase in both aortic and sinus nerve activity can be seen in the second frame.

Figure 1

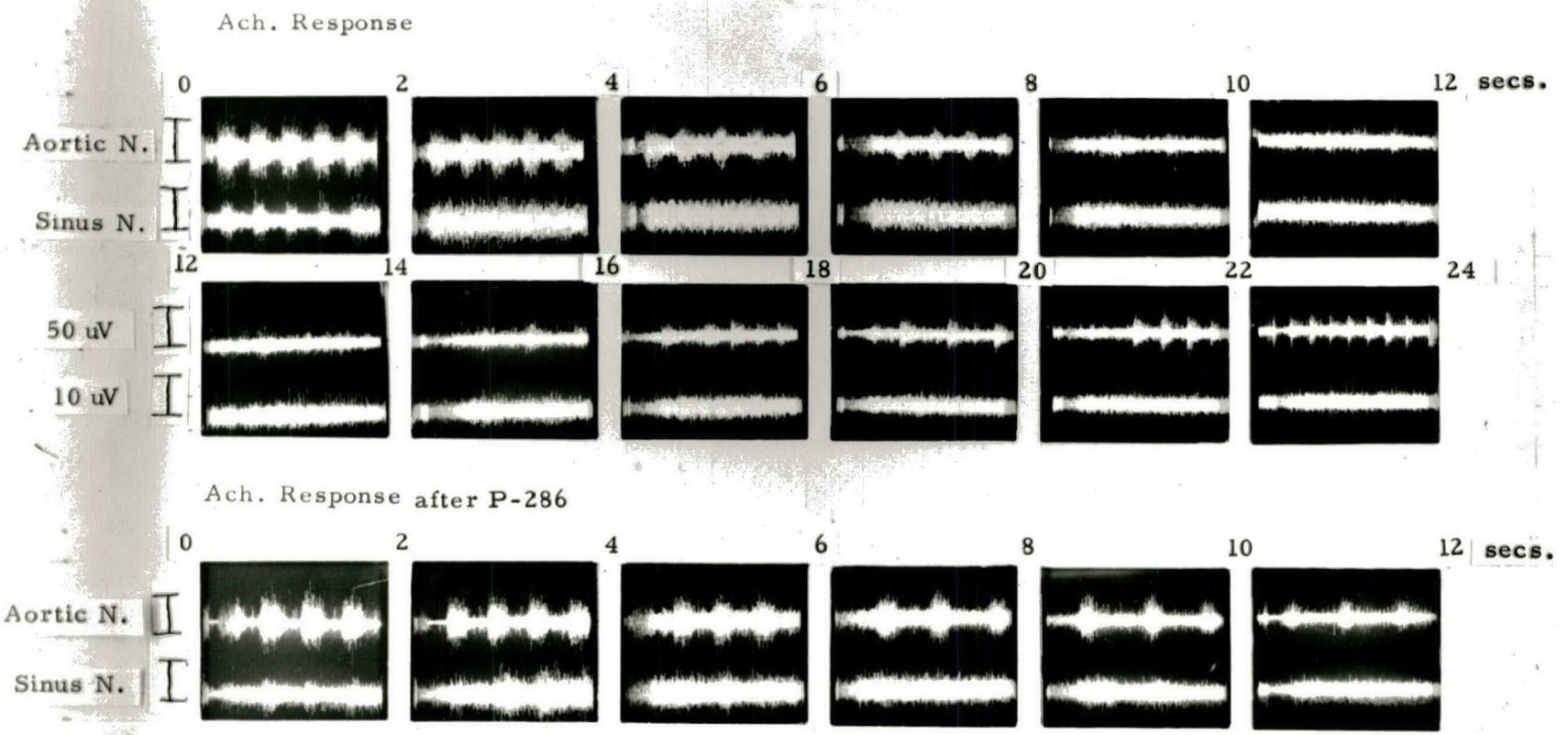

Ach. Response
0
2
4
6
8
10
12
secs.
Aortic N.
Sinus N.
12
14
16
18
20
22
24
50 uV
10 uV
Ach. Response after P-286
0
2
4
6
8
10
12
secs.
Aortic N.
Sinus N.

Figure 2 Electrical activity in the sinus nerve and blood pressure response after the injection of acetylcholine 1 mg/kg I.V. before and after P-286 6 mg/kg I.V. and 12 mg/kg I.V.

The upper two rows represent the acetylcholine response before 6 mg/kg of P-286. The upper beam is sinus nerve activity. The bottom beam is the arterial blood pressure. The initial activity starts at the end of the first frame. The initial increase in activity in the third row begins at the end of the first frame. The initial increase in activity in the bottom row begins in the third frame.

Figure 2

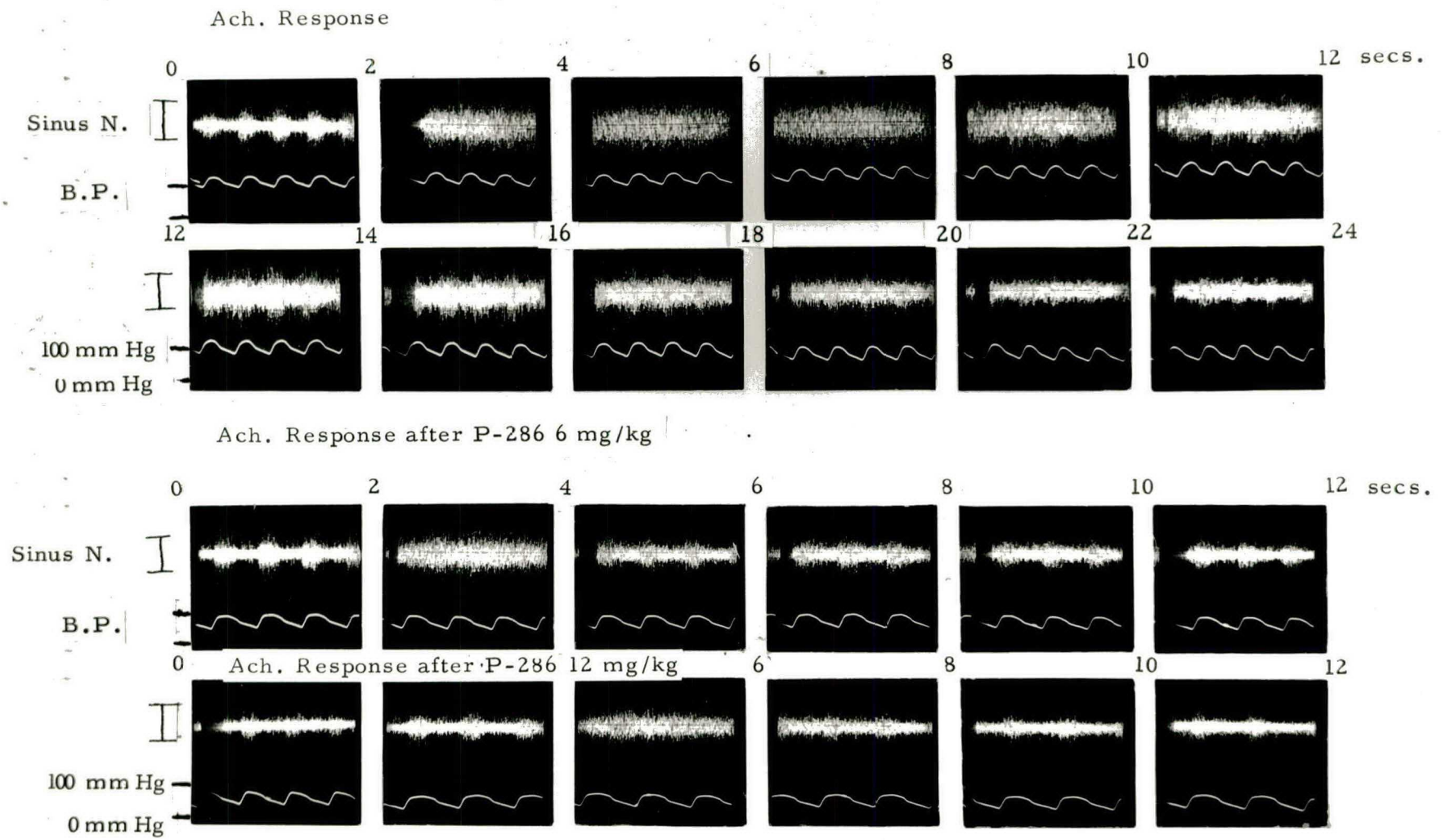

Ach. Response
0
2
4
6
8
10
12 secs.
Sinus N.
B.P.
12
14
16
18
20
22
24
100 mm Hg
0 mm Hg
Ach. Response after P-286 6 mg/kg
0
2
4
6
8
10
12 secs.
Sinus N.
B.P.
0
Ach. Response after P-286 12 mg/kg
6
8
10
12
100 mm Hg
0 mm Hg

Figure 3 Electrical activity in the superior cervical preganglionic sympathetic nerve (S.C.S.N.) and aortic nerve during the response to acetylcholine 1 mg/kg before and after P-286 6 mg/kg.

In the top row the increase in aortic nerve activity starts at the beginning of the fourth frame (or after a lapse of 6 seconds). The increase in sympathetic activity begins in the middle of the same frame.

In the third row, increased electrical activity in the aortic nerve begins at the end of the third frame. Increased sympathetic activity begins in the fourth frame.

Figure 3

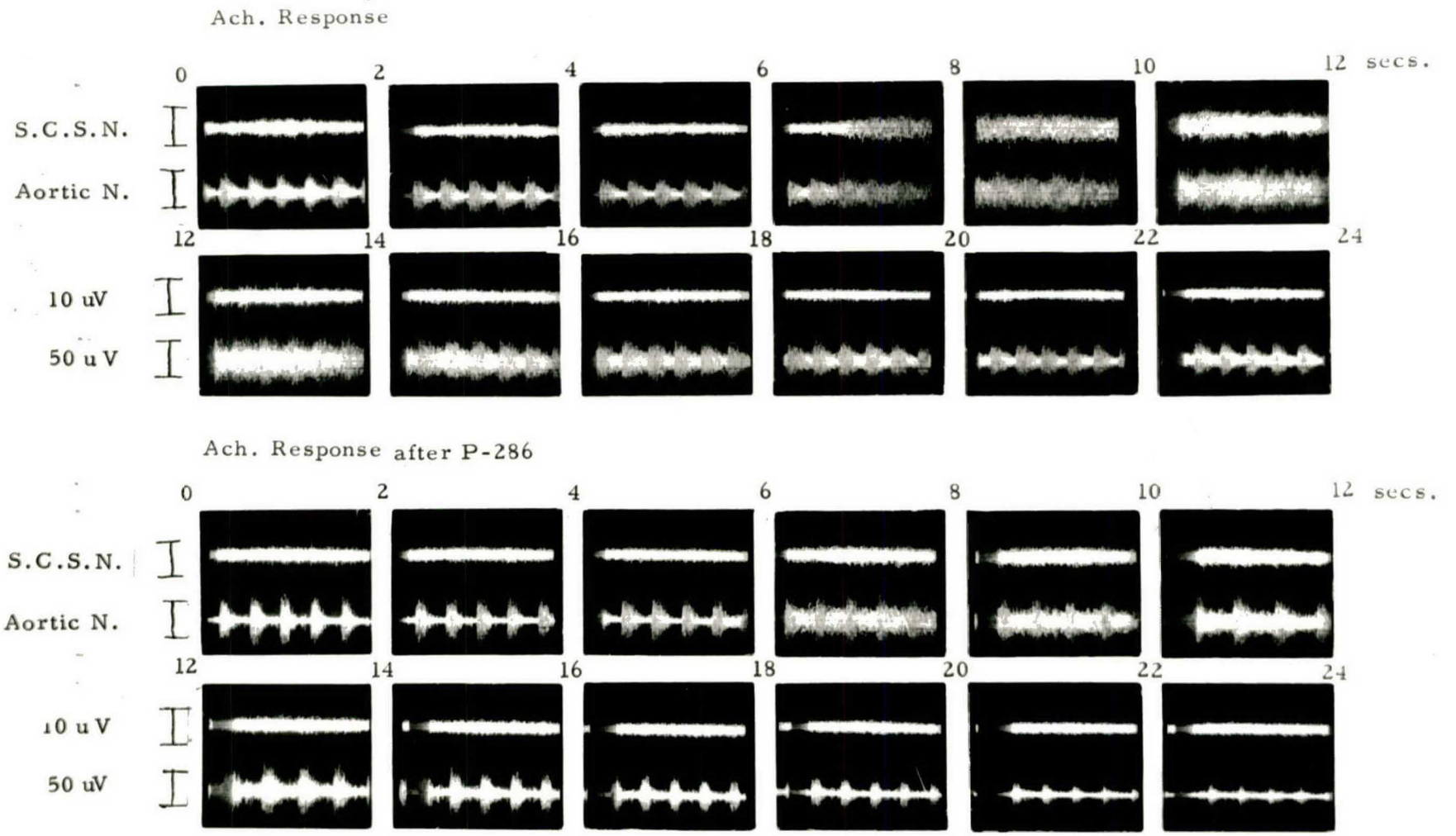

Ach. Response
0
2
4
6
8
10
12 secs.
S.C.S.N.
Aortic N.
12
14
16
18
20
22
24
10 uV
50 uV
Ach. Response after P-286
0
2
4
6
8
10
12 secs.
S.C.S.N.
Aortic N.
12
14
16
18
20
22
24
10 uV
50 uV

Figure 4 Blood pressure and respiratory responses to acetylcholine 1 mg/kg I.V. before and after P-286 6 mg/kg I.V.

Figure 4

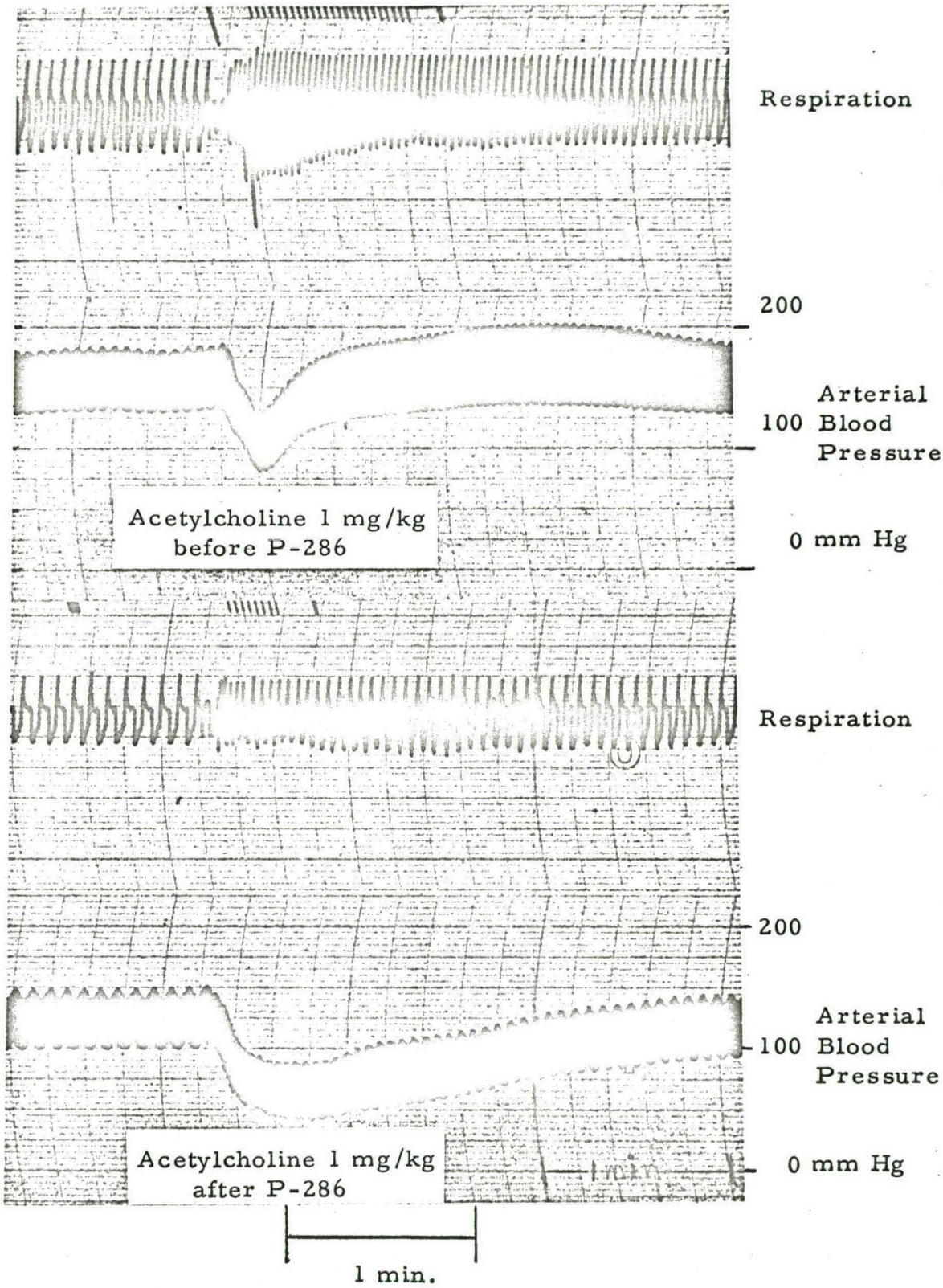

Respiration
200
Arterial
100 Blood
Pressure
Acetylcholine 1 mg/kg
before P-286
0 mm Hg
Respiration
200
Arterial
100 Blood
Pressure
Acetylcholine 1 mg/kg
after P-286
1 min
0 mm Hg
1 min.

Figure 5 Blood pressure and respiratory responses to acetylcholine 1 mg/kg I.V. before and after P-286 6 mg/kg I.V.

Figure 5

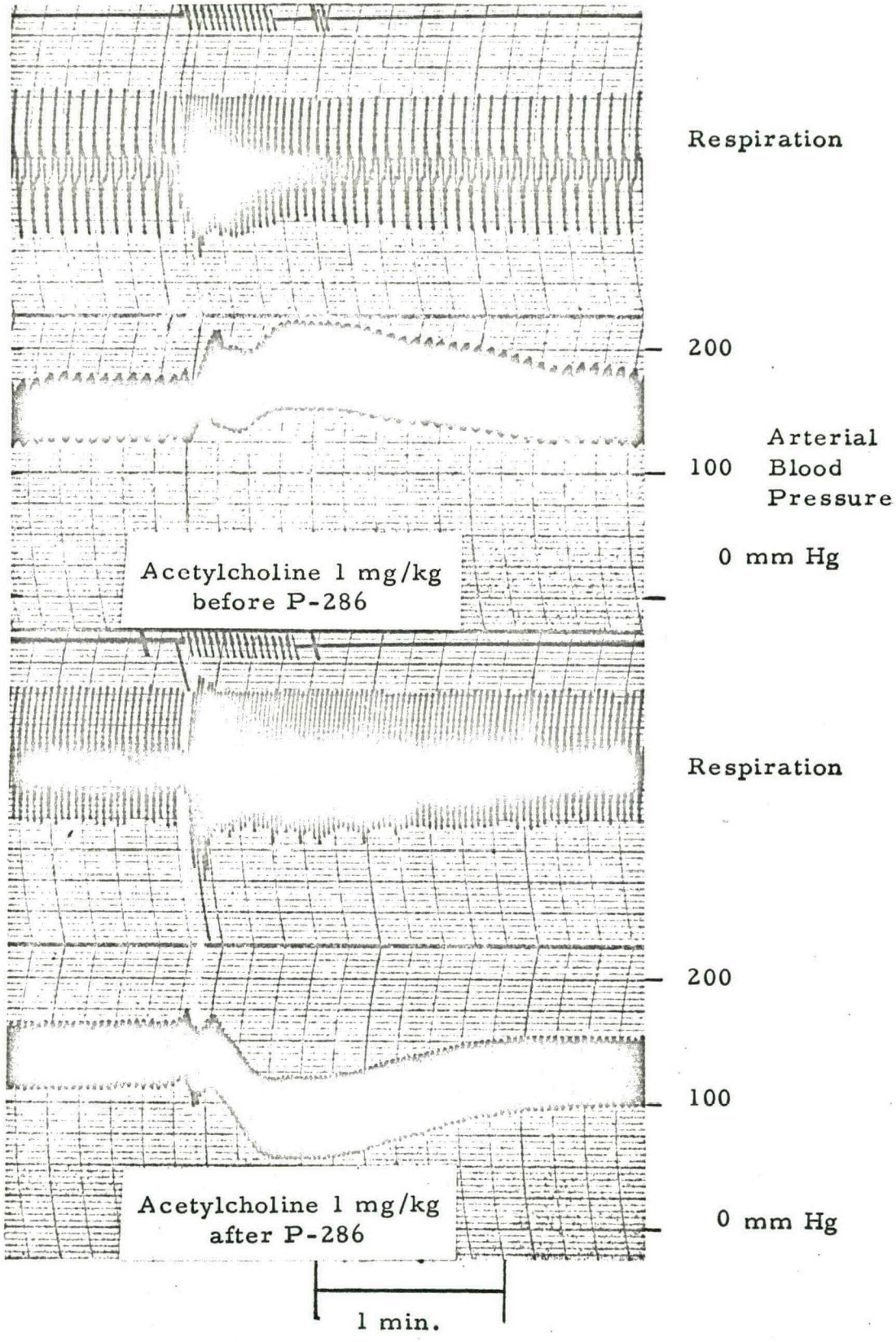

Respiration
200
Arterial
Blood
Pressure
100
0 mm Hg
Acetylcholine 1 mg/kg
before P-286
Respiration
200
100
Acetylcholine 1 mg/kg
after P-286
0 mm Hg
1 min.

Figure 6 Electrical activity in the superior cervical preganglionic sympathetic nerve (S.C.S.N.) during the response to DMPP 40 ug/kg I.V.

In the top row, increased activity seems to occur simultaneously in both nerves at the beginning of the second frame. In the bottom row after P-286, increased activity is apparent only in the aortic nerve starting in the second frame.

Figure 6

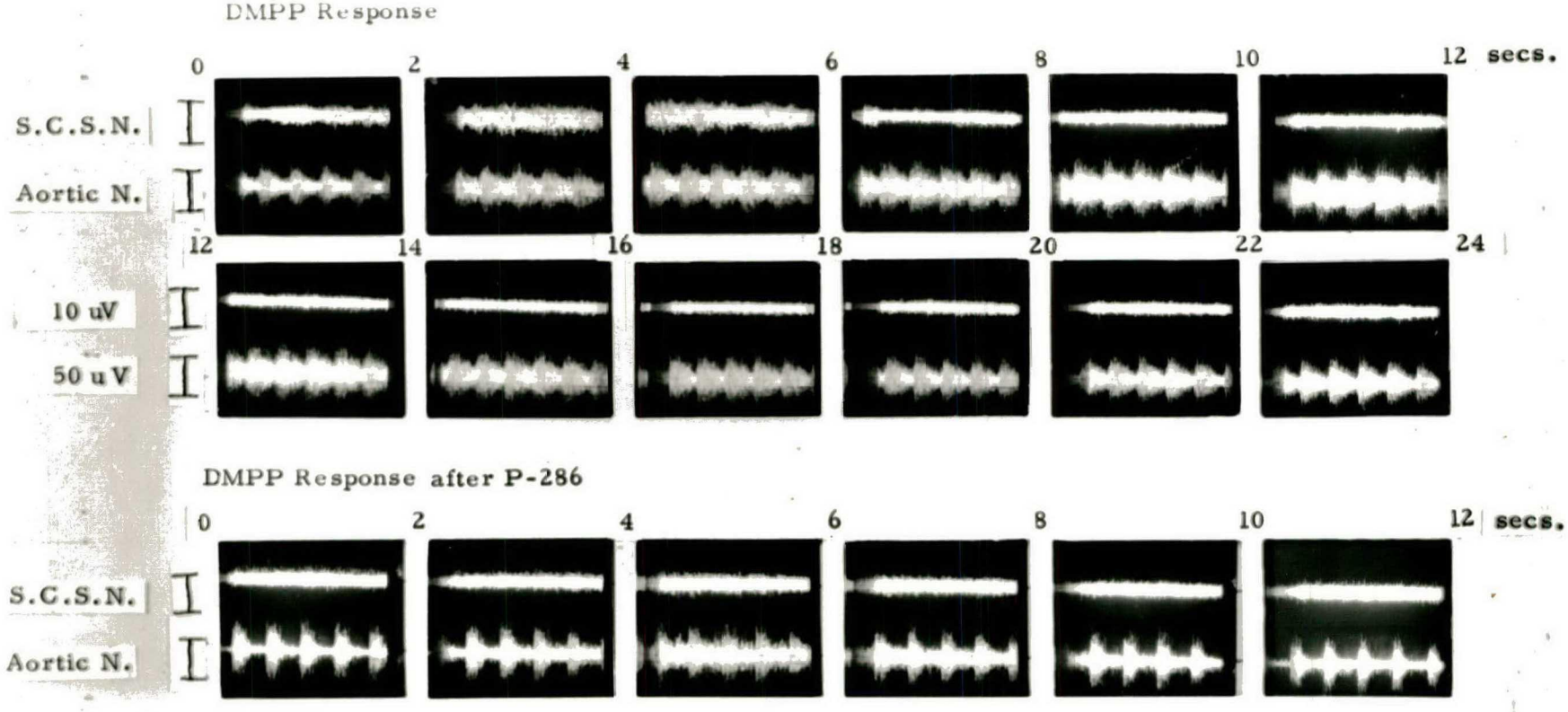

DMPP Response
0
2
4
6
8
10
12 secs.
S.C.S.N.
Aortic N.
12
14
16
18
20
22
24
10 uV
50 uV
DMPP Response after P-286
0
2
4
6
8
10
12 secs.
S.C.S.N.
Aortic N.

Figure 7 Blood pressure and respiratory responses to DMPP 40 ug/kg I.V. before and after P-286 6 mg/kg I.V.

Figure 7

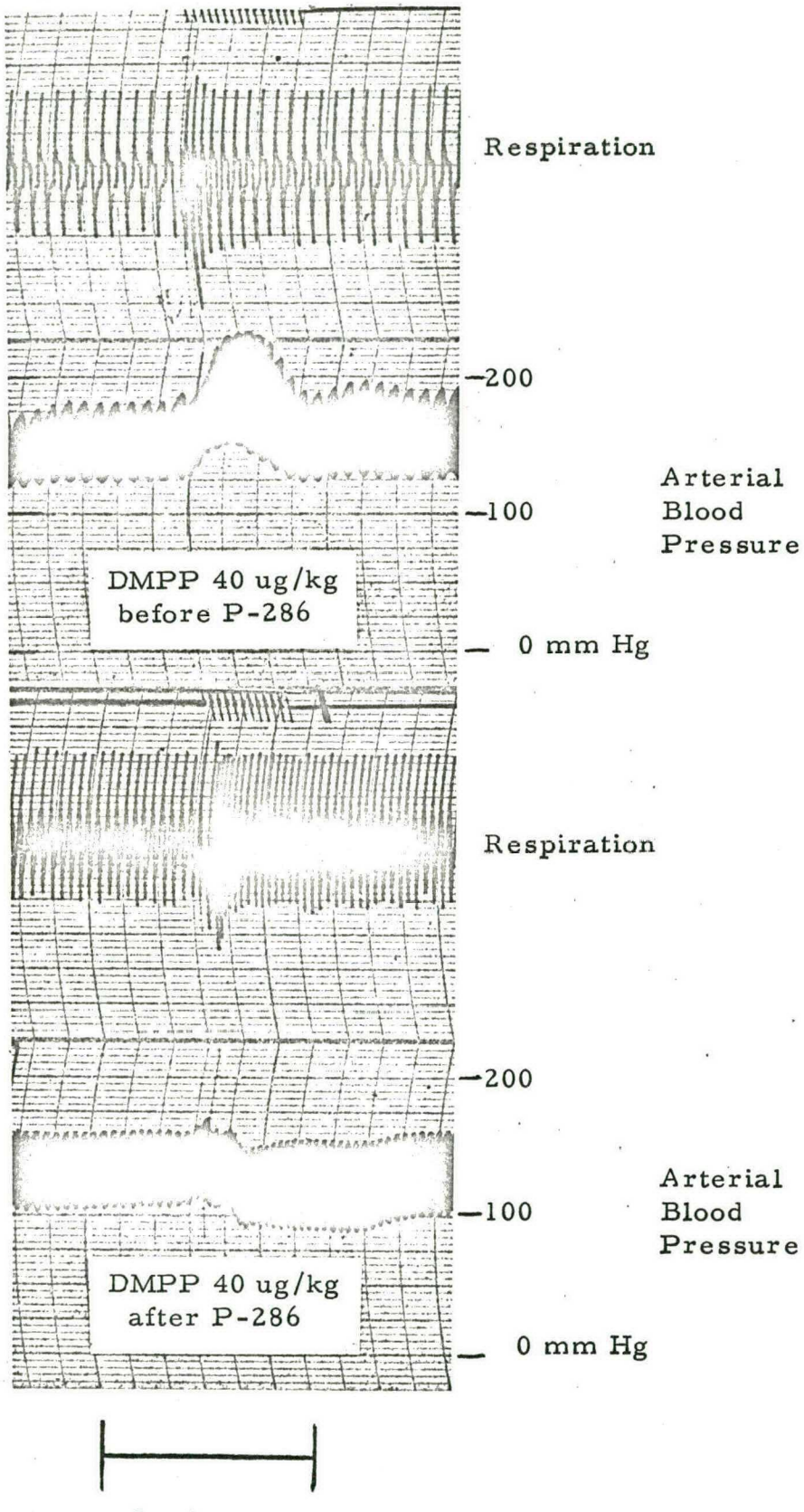

Respiration
200
100
Arterial
Blood
Pressure
DMPP 40 ug/kg
before P-286
0 mm Hg
Respiration
200
100
Arterial
Blood
Pressure
DMPP 40 ug/kg
after P-286
0 mm Hg
1 min.

Figure 8 Electrical activity in the sinus and aortic nerves during the response to sodium cyanide 50 ug/kg I.V. before and after P-286 6 mg/kg I.V.

In the top row, the increase in sinus nerve activity can be seen near the end of the first frame. The increase in aortic nerve activity is nearly obscured by the larger baroreceptor activity and is equivocal. After P-286, increase in sinus nerve activity can be seen in the second frame of the third row. In the case of the aortic nerve, again it is difficult to describe any increase in activity.

Figure 8

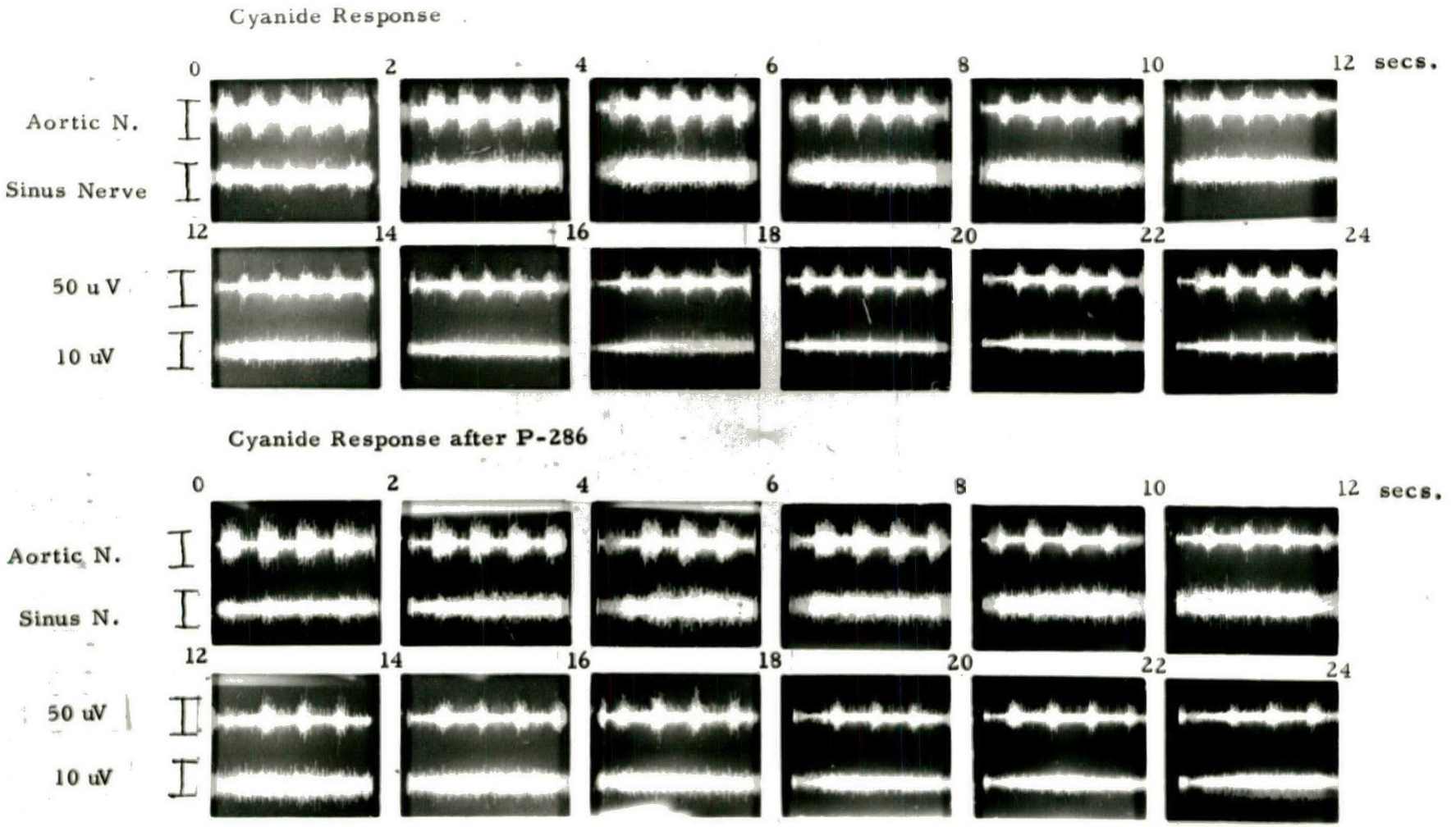

Cyanide Response
0
2
4
6
8
10
12 secs.
Aortic N.
Sinus Nerve
12
14
16
18
20
22
24
50 u V
10 uV
Cyanide Response after P-286
0
2
4
6
8
10
12 secs.
Aortic N.
Sinus N.
12
14
16
18
20
22
24
50 uV
10 uV

Figure 9

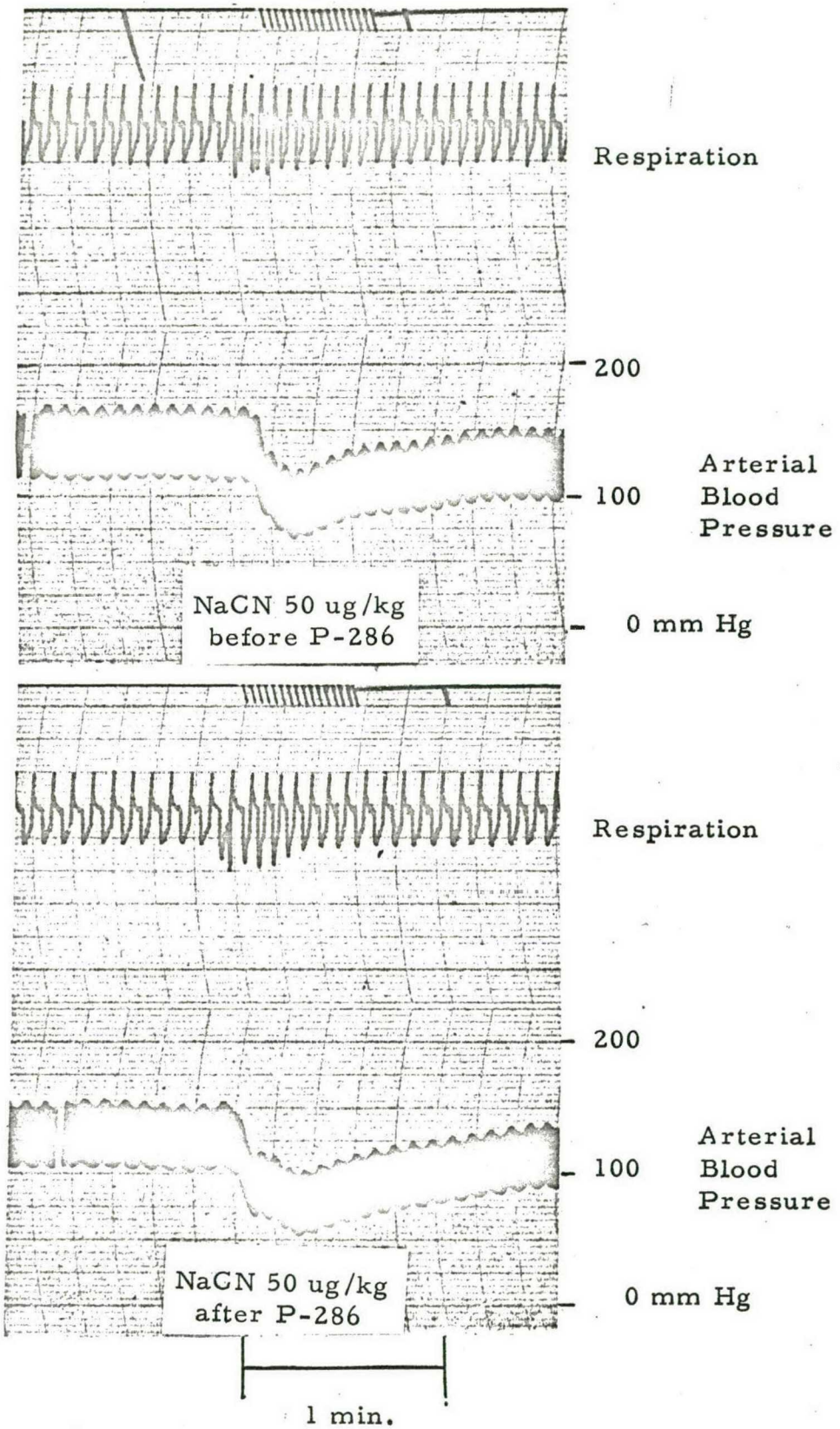

Respiration
200
100
Arterial
Blood
Pressure
NaCN 50 ug/kg
before P-286
0 mm Hg
Respiration
200
100
Arterial
Blood
Pressure
NaCN 50 ug/kg
after P-286
0 mm Hg
1 min.

Figure 10 Blood pressure and respiratory responses to sodium cyanide 50 ug/kg I.V. before and after P-286 6 mg/kg I.V.

Figure 10

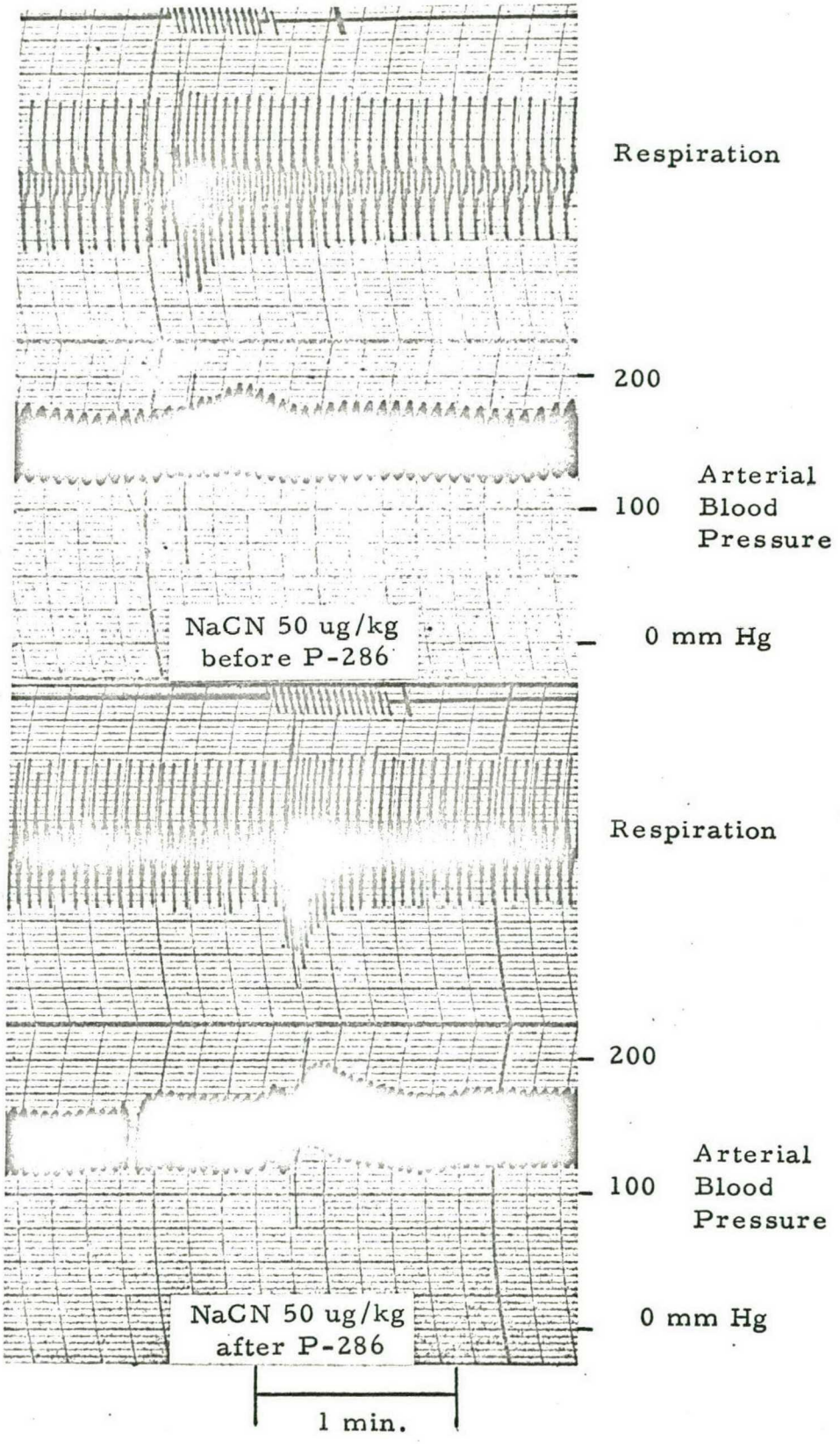

Respiration
200
Arterial
Blood
Pressure
100
NaCN 50 ug/kg
before P-286
0 mm Hg
Respiration
200
Arterial
Blood
Pressure
100
NaCN 50 ug/kg
after P-286
0 mm Hg
1 min.

Figure 11 Electrical activity in the sinus nerve and blood pressure response after the injection of sodium cyanide 50 ug/kg I.V. before and after P-286 6 mg/kg I.V.

Figure 11 Electrical activity in the sinus nerve and blood pressure response after the injection of sodium cyanide 50 ug/kg I.V. before and after P-286 6 mg/kg I.V. and 12 mg/kg I.V.

The upper row represents the increase in the sinus nerve and the blood pressure response during sodium cyanide before P-286; the second row after 6 mg/kg of P-286; the third row after a total of 12 mg/kg of P-286. The increase in activity in the top row is synchronized with the second systole of the first frame. Nerve activity is increased at the end of the first frame, and in the bottom row in the second frame.

Figure 11

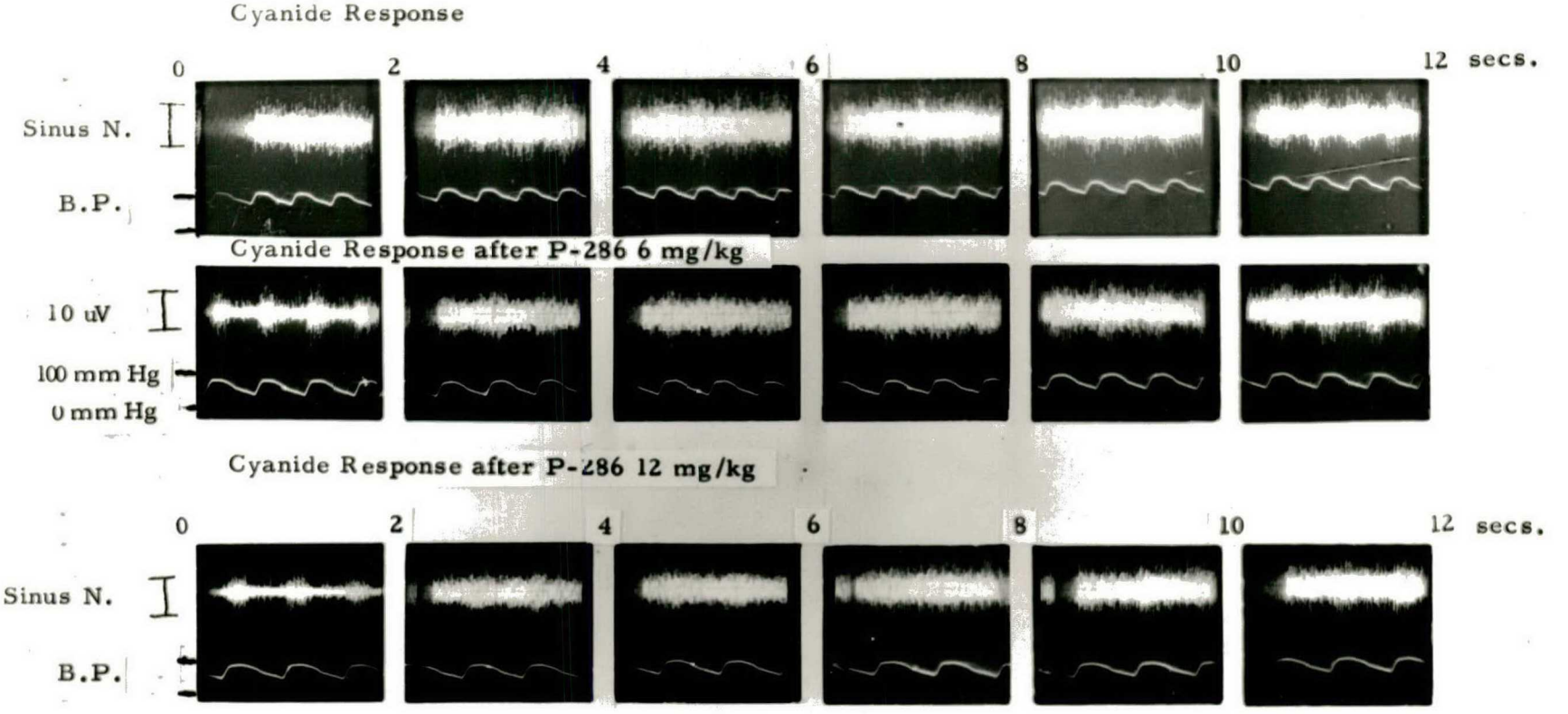

Cyanide Response
0
2
4
6
8
10
12 secs.
Sinus N.
B.P.
Cyanide Response after P-286 6 mg/kg
10 uV
100 mm Hg
0 mm Hg
Cyanide Response after P-286 12 mg/kg
0
2
4
6
8
10
12 secs.
Sinus N.
B.P.

Figure 12 Blood pressure and respiratory responses to animal breathing carbon dioxide 90 per cent and oxygen 10 per cent for 60 seconds before and after P-286 6 mg/kg I.V.

Figure 12

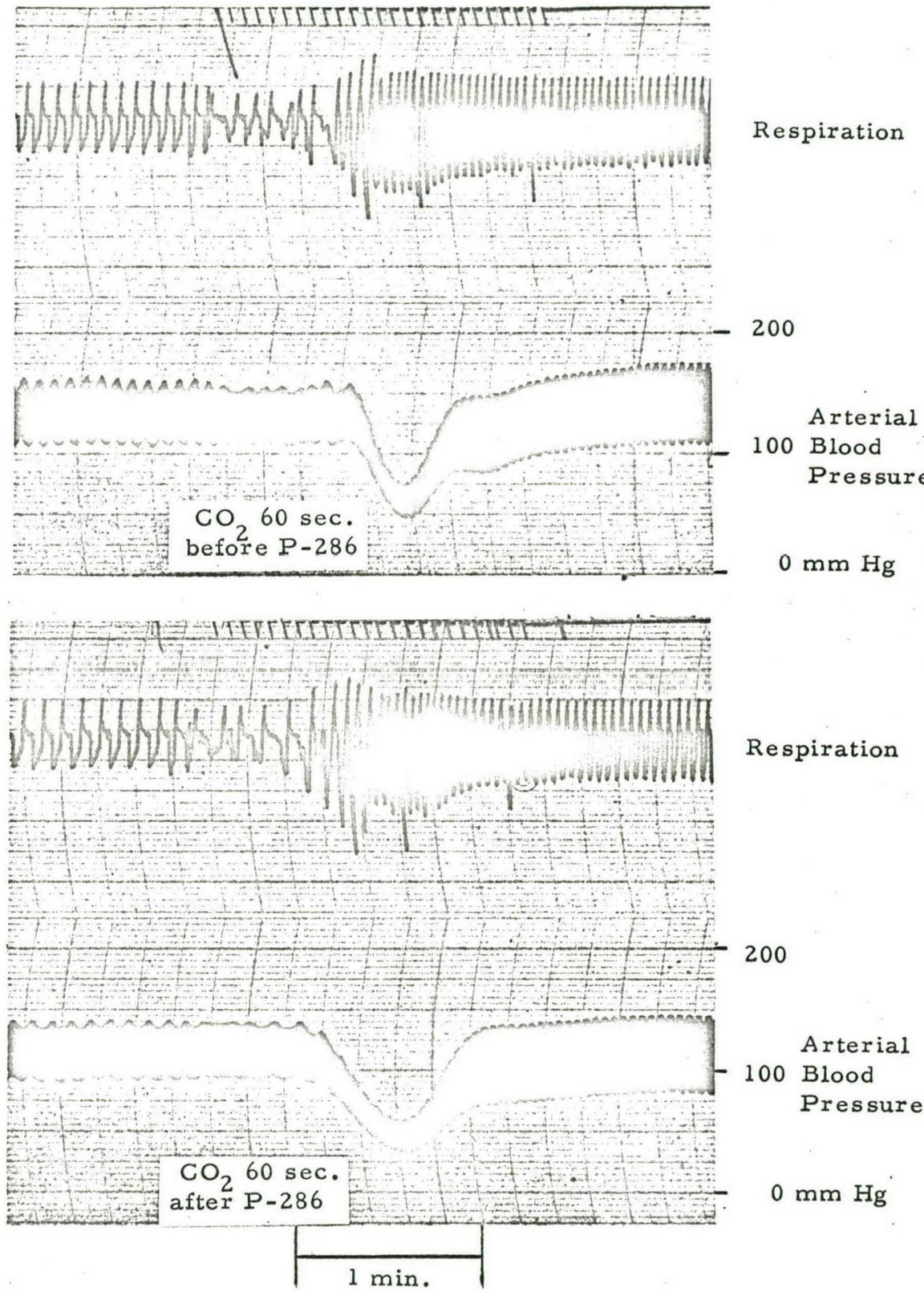
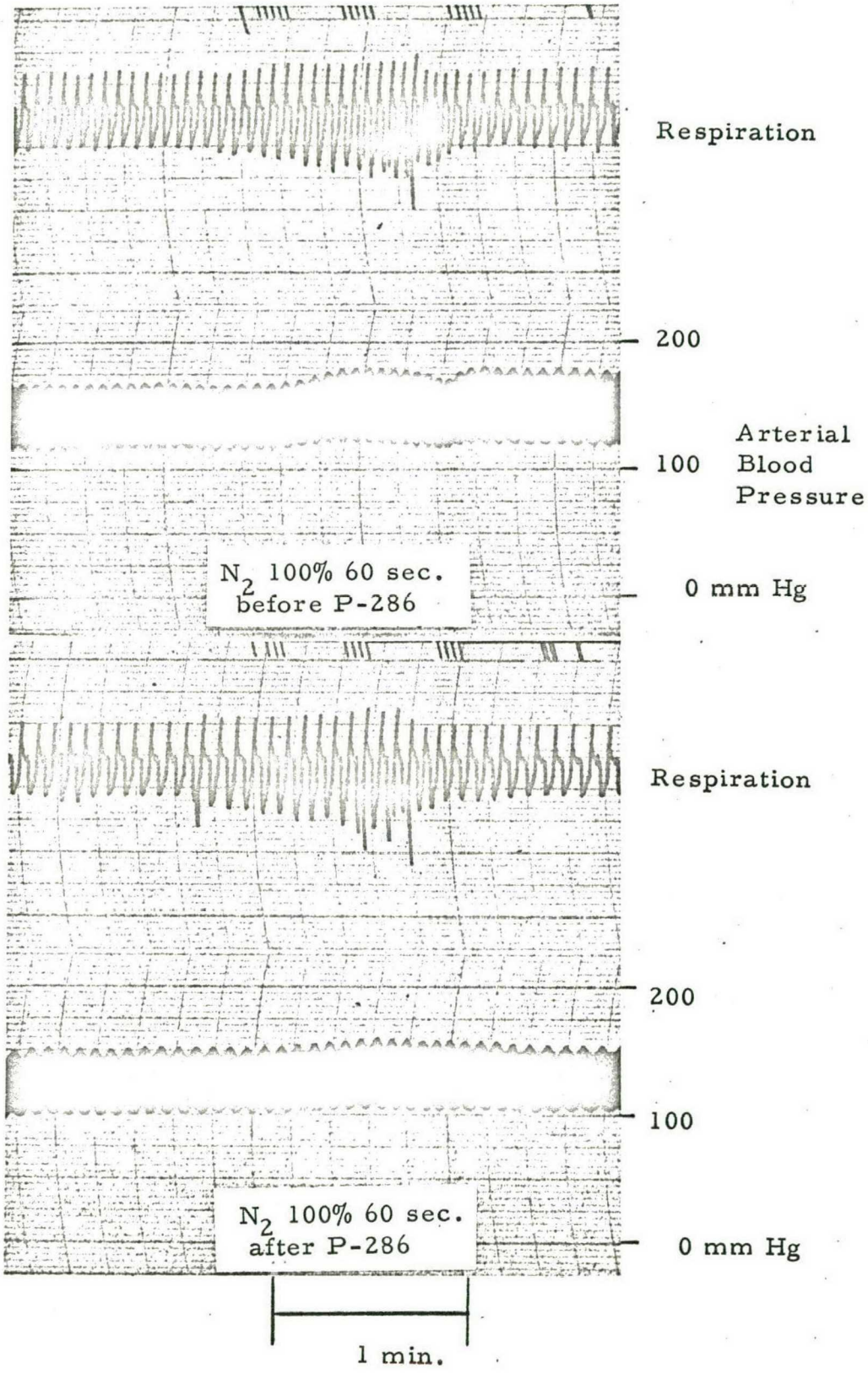

Respiration
200
Arterial
100 Blood
Pressure
0 mm Hg
CO_2 60 sec.
before P-286
Respiration
200
Arterial
100 Blood
Pressure
0 mm Hg
CO_2 60 sec.
after P-286
1 min.

Figure 13 Blood pressure and respiratory responses to animal breathing nitrogen 100 per cent for 60 seconds before and after P-286 6 mg/kg I.V.

Figure 13


Respiration
200
100
Arterial
Blood
Pressure
N_2 100% 60 sec.
before P-286
0 mm Hg
Respiration
200
100
N_2 100% 60 sec.
after P-286
0 mm Hg
1 min.

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