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Circulatory accessibility of airways slowly adapting mechanoreceptors having known location

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Fisiologia. — *Circulatory accessibility of airways slowly adapting mechanoreceptors having known location* (*). Nota di FRANCA BRAMBILLA SANT'AMBROGIO e GIUSEPPE SANT'AMBROGIO (**), presentata (***) dal Socio R. MARGARIA.

Riassunto. — Abbiamo studiato l'accessibilità, dal circolo sistemico (C.S.) e polmonare (C.P.), di recettori a lento adattamento, localizzati nelle vie aeree. Gli esperimenti sono stati eseguiti in 31 cani anestetizzati, vagotomizzati, paralizzati e artificialmente ventilati a gabbia toracica aperta. Abbiamo misurato la pressione arteriosa nel circolo sistemico, la pressione tracheale, il CO_2 nell'aria espirata e abbiamo registrato potenziali d'azione unitari da filamenti sottili separati dal nervo vago. Detti recettori sono stati localizzati broncoscopicamente o per sondaggio diretto nelle vie aeree extra-polmonari e dissezionando il parenchima polmonare, alla fine dell'esperimento, nel caso di quelli intrapolmonari. Per stimolare i recettori è stata usata la veratridina, che non modifica la meccanica polmonare, in dosi di 10–15 μg/kg, iniettata o nell'atrio destro o nell'atrio sinistro. Undici dei 15 recettori studiati e localizzati nella trachea o nel bronco principale sono stati stimolati dalla veratridina in 7.27 ± 0.66 sec quando somministrata in via C.S. e in 17.10 ± 3.07 sec quando somministrata via C.P. Quattro recettori non sono stati modificati nella loro scarica. I 16 recettori localizzati in vie aeree di varie dimensioni (diametro 0.3–5.0 mm) all'interno del polmone sono stati invece tutti stimolati dalla veratridina e in un tempo più breve quando questa veniva iniettata via C.P.: tempo medio 5.25 ± 0.72 sec rispetto ad un tempo medio di 15.54 ± 1.63 sec per via C.S. Questi risultati indicano che le vie aeree intrapolmonari, anche se di diametro ragguardevole, vengono preferenzialmente perfuse attraverso la circolazione polmonare. Il presumere quindi che una maggiore accessibilità attraverso la C.P. sia indicativa di una localizzazione in vie aeree molto periferiche e, viceversa, una accessibilità maggiore attraverso la C.S. presuma una locazione in vie aeree più centrali sembra ingiustificato. Inoltre il fatto che i recettori intrapolmonari siano più facilmente accessibili attraverso la C.P. suggerisce che queste terminazioni nervose possono essere modificate dalle sostanze presenti soprattutto nel sangue venoso misto.

INTRODUCTION

The lungs have a dual blood supply: 1) the pulmonary circulation, whose main purpose is to convey the venous blood to the gas exchanging zone in the alveoli, and is classically thought to perfuse only the airways peripheral to the terminal bronchioles and 2) the bronchial circulation, carrying arterial blood, which under normal circumstances has only a nutritional purpose and supplies the tracheo-bronchial tree as far as the respiratory bronchioles (Miller, 1973; Daly and Hebb, 1966).

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These two circulations have connections at the capillary and venous level and, in the normal lung of man and some other species, also precapillary anastomoses have been anatomically demonstrated (Verloop, 1948; Rakshit, 1949; Nagaishi, 1972).

Furthermore, lung autotransplantation and hilar stripping do not usually lead to bronchial necrosis even though the bronchial circulation is interrupted and its reestablishment takes at least two weeks (Fisher *et al.*, 1970). These findings suggest that the pulmonary circulation is capable of perfusing also large airways (Ellis *et al.*, 1951; Hughes *et al.*, 1954; Fisher *et al.*, 1970; Pinsker *et al.*, 1979).

It is very common to infer the location of a given type of airways receptor by its accessibility from either the systemic or the pulmonary circulation. If a drug known to modify the receptor behaviour does so more rapidly when injected into the systemic circulation the receptor is considered to be located in airways central to the terminal bronchioles; conversely it is thought to belong to very peripheral airways when it is reached with a shorter delay from the pulmonary circulation (Paintal, 1955; Coleridge *et al.*, 1965; Paintal, 1969; Armstrong and Luck, 1974; Russell and Trenchard, 1980).

In view of the present knowledge on the interrelations between the two circulations, this approach deserves to be experimentally evaluated.

The purpose of this study is that of determining the preferential accessibily of airways slowly adapting mechanoreceptors, *of known location*, from either the systemic or the pulmonary circulation, to clarify the functional interrelations between these two vascular beds and to assess the validity of the pharmacological approach in the localization of pulmonary receptors.

METHODS

The experiments have been performed on dogs, in the 10–15 kg weight range, anesthetized with sodium pentobarbital (initial dose 30 mg/kg).

The trachea was cannulated just below the cricoid cartilage.

A polyethylene catheter was inserted through the femoral vein and positioned at the level of the right atrium for administration of veratridine into the pulmonary circulation; the same catheter was also used for injections of other drugs.

For monitoring systemic blood pressure a second catheter was placed in the femoral artery and connected to a Statham pressure transducer.

The catheter for administering veratridine into the systemic circulation was placed directly in the left auricle.

The dog was then artificially ventilated through a Harvard respirator (tidal volume and frequency kept as close as possible to control conditions and matched in such a way to obtain an end-tidal P_{CO_2} of about 40 mm Hg), paralyzed with gallamine (5 mg/kg) and the chest was widely opened by splitting the sternum in the mid-line; 3–4 cm H_2O end-expiratory positive pressure was maintained.

The tracheal pressure was monitored through a side arm of the tracheal cannula connected to a Statham pressure transducer.

Rectal temperature and end-tidal CO_2 (Beckman infra-red analyzer) were also monitored.

Both vagus nerves were cut high in the neck.

The peripheral cut end of the right vagus nerve was placed on a dissecting tray, flooded with paraffin oil, and desheathed; thin filaments were separated using a pair of watchmaker forceps, with the aid of a Zeiss binocular microscope.

The vagal filaments were placed on a pair of platinum electrodes connected to an AC coupled amplifier; the signal was displaced on a Tektronix oscilloscope, in parallel with a loudspeaker.

The dissection of the filaments was continued until one was found containing only one active fiber that was recognized as originating from a stretch receptor by its regular respiratory modulation and its slow rate of adaptation to a maintained pressure.

Action potentials, tracheal pressure and blood pressure were recorded on a Honeywell Visicorder and on a Hewlett Packard tape recorder. Firing rate of the receptor (electronically computerized) was also recorded on a Gould-Brush oscillograph, together with tracheal and arterial pressure.

Veratridine was then injected (10–15 μg/kg) into either the right (R.H.) or left heart (L. H.) and flushed with 1.5 ml heparinized saline. Fifteen or twenty minutes were allowed to elapse before the second injection was administered, in order to allow the receptor to resume its control rate of discharge and the arterial pressure to return to its pre-injection value.

The two routes of administration, systemic and pulmonary circulations, were compared considering the delay between injection and onset of stimulation and the increase in firing rate of the receptor.

The extra-pulmonary receptors were localized either by activating them with the cuff of a Foley catheter inserted in the lumen of the trachea, or with the aid of a bronchoscope which could directly stimulate the endings.

The intraparenchymal receptors were localized in one of the lobes by direct manipulation of the lung surface, at the end of the experiment. The dog's heart was then clamped to stop the circulation, the lung parenchyma cut away in small pieces, starting from the periphery, while a flow of air maintained the airways properly distended in order to activate the receptor. The exposed cut surface was probed and a catheter passed through the transversally cut bronchi so its tip could eventually stimulate the ending.

The size of the airways was measured, with a suitable ruler, either by eye or with the aid of a microscope. Its generation order was determined visually after having dissected out the remaining portion of the lobe.

Veratridine is an ester that belongs to the group of veratrum alkaloids. These substances are known to stimulate receptors in the cardiovascular and respiratory systems that reflexly cause hypotensions and apnea (Benforado, 1967).

Intravenous injections of veratridine greatly increase the rate of firing of pulmonary slowly adapting mechanoreceptors (Dawes *et al.*, 1951; Paintal, 1964).

This drug, at the concentrations used in this research, does not have any effect on lung mechanics, a change of which would affect receptor discharge interfering with the direct effect of the drug on the ending.

RESULTS

Thirty-one airways slowly adapting mechanoreceptors of known location have been studied.

Eleven of the 15 tracheal receptors considered were stimulated by injection of veratridine into the left heart with a mean delay of 7.44 ± 0.80 seconds. Four of them were also activated from the right heart, but with a considerably longer delay (mean 17.12 ± 3.96 sec). The remaining 4 were

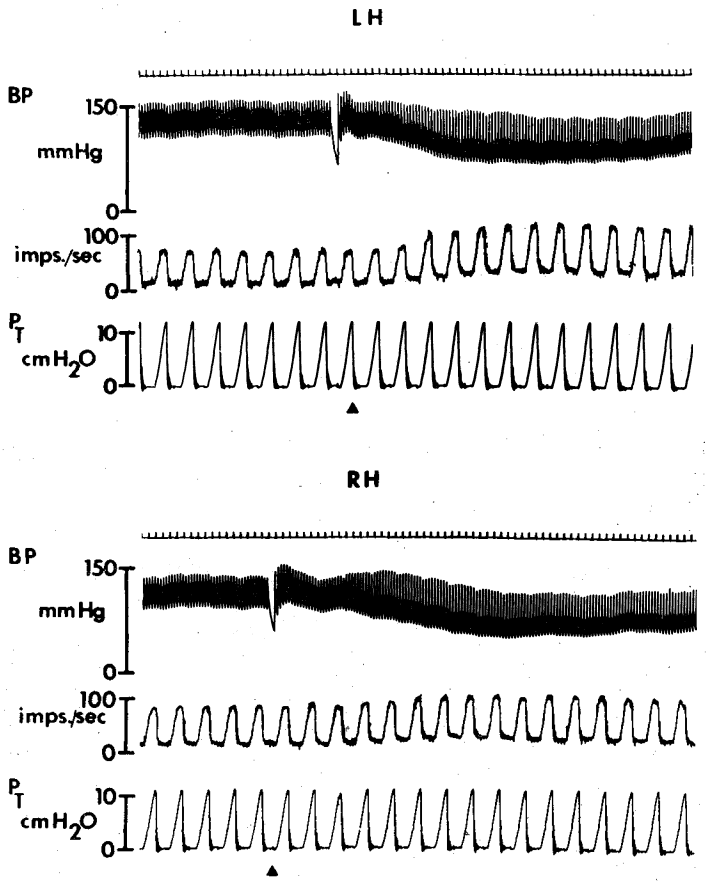

Fig. 1. – Effect of veratridine injected (at arrows) into the left (LH) and right heart (RH) on a tracheal receptor. LH injection stimulated the receptor in 6 sec, RH injection sensitized the ending in 13.5 sec. In each record, from top to bottom: time marker = 1 sec; arterial blood pressure (BP); rate of discarge of the receptor (imps/sec); tracheal pressure (P_T).

not affected either by left or by right heart injections. A two tailed t test was performed and the statistical difference between the delays due to R. H. and L. H. injections found to be highly significant ($P < 0.01$). Fig. 1 shows an example of a tracheal receptor.

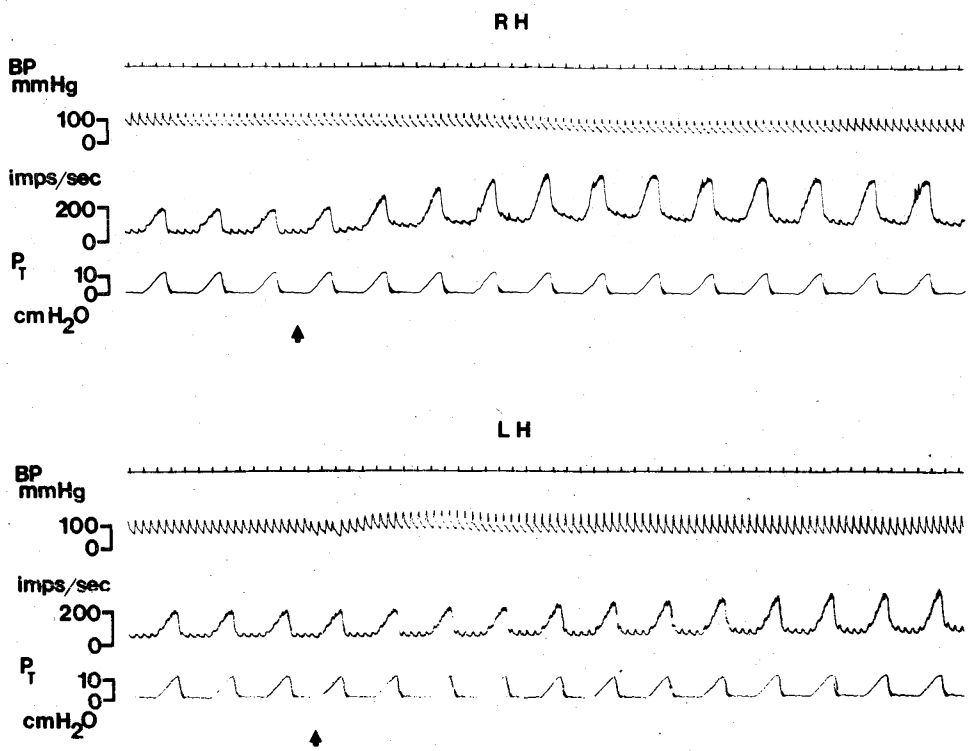

Fig. 2. – Effect of veratridine injected (at arrows) into the left (LH) and right heart (RH) on a receptor localized in an intraparenchymal bronchus. LH injection caused sensitization of the receptor in 10 sec, RH injection stimulated the ending in 3.5 sec. In each record, from top to bottom: time marker = 1 sec; arterial blood pressure (BP); rate of discharge of the receptor (imps/sec); tracheal pressure (P_T).

Two receptors, located in the main stem bronchus, were reached through the systemic circulation respectively in 7 and 6 sec. Veratridine injected into the right heart activated only one of them and with a considerably longer delay (17 sec).

The remaining 16 endings, localized in bronchi of various size (0.3–5.0 mm in diameter), were readily stimulated by veratridine injected into the pulmonary circulation (mean delay 5.25 ± 0.72 sec). Fourteen of them were stimulated also when the drug was injected into the systemic circulation, but with a much longer delay (mean 15.54 ± 1.63 sec). The statistical difference between the two groups was highly significant ($P < 0.01$).

Fig. 2 shows an example of the effect of veratridine on one of the intra-parenchymal receptors.

The delays and degrees of activation for each of the receptors studied are reported in Table I.

TABLE I.

Accessibility of tracheo-bronchial receptors through the systemic (Left Heart) and pulmonary (Right Heart) circulations.

The activity of each receptor has been measured as the number of action potentials per pump cycle. The delay has been calculated as the time from the injection to the onset of stimulation. The increase in activity is shown as % of the control situation and is the maximum reached.

	L. H.		R. H.	
	Delay	Increase in activity	Delay	Increase in activity
Tracheal receptors:				
1.	5.5 "	156%	—	—
2.	—	—	—	—
3.	5.5 "	147%	13.0 "	125%
4.	—	—	—	—
5.	5.5 "	114%	13.5 "	116%
6.	7.5 "	140%	29.0 "	112%
7.	—	—	—	—
8.	6.5 "	148%	13.0 "	112%
9.	11.0 "	208%	—	—
10.	11.5 "	199%	—	—
11.	—	—	—	—
12.	5.5 "	309%	—	—
13.	8.5 "	116 %		—
Bronchial receptors (main stem bronchus)				
1.	7.0 "	297%	17.0 "	393%
2.	6.0 "	150%	—	—

Segue: TABLE I.

	L. H.		R. H.	
	Delay	Increase in activity	Delay	Increase in activity
Bronchial receptors (intraparenchymal):				
1 – diameter: 5.0 mm	9.5 "	140%	3.5 "	198%
2 – » 4.5 mm	14.5 "	132%	6.0 "	374%
3 – » 4.4 mm	22.0 "	121%	6.0 "	149%
4 – » 4.0 mm	10.0 "	190%	2.5 "	676%
5 – » 3.4 mm	21.0 "	107%	10.0 "	115%
6 – » 3.0 mm	—	—	12.0 "	122%
7 – » 3.0 mm	8.5 "	722%	3.5 "	632%
8 – » 2.5 mm	23.0 "	109%	4.0 "	285%
9 – » 1.5 mm	15.0 "	111%	4.5 "	149%
10 – » 1.0 mm	25.0 "	110%	10.0 "	128%
11 – » 0.9 mm	—	—	4.5 "	148%
12 – » 0.7 mm	16.0 "	141%	3.5 "	328%
13 – » 0.6 mm	11.0 "	135%	3.0 "	139%
14 – » 0.5 mm	9.0 "	143%	4.0 "	555%
15 – » 0.4 mm	23.0 "	165%	3.5 "	1,050%
16 – » 0.3 mm	10.0 "	172%	3.5 "	195%

DISCUSSION

The tracheal receptors have been considered in this study for obtaining some basic information to be used as points of reference.

The trachea is undoubtedly perfused only by the systemic circulation, therefore the delay between L. H. injection of veratridine and subsequent stimulation represents the time required for a direct effect on the ending. This delay has two components: the circulatory time from the left heart to the capillaries surrounding the receptor, and the diffusion time from the capillaries to the receptor itself.

The delay for the stimulation of the tracheal receptors by veratridine injected into the R. H. is longer for two concurrent reasons: 1) the additional circulatory time through the pulmonary circulation and 2) a longer diffusion time due to a lower concentration of veratridine into the blood perfusing the receptor site, because of its dilution in both heart chambers.

Four tracheal receptors escaped stimulation by both routes; this might be indicative of either a different location within the trachealis muscle, in a poorly perfused area, or of an intrinsic insensitivity to the drug injected.

The ending localized in the main stem bronchus behaved like the majority of the tracheal receptors, being preferentially activated through the systemic circulation. This finding is in accordance with the observations by Pinsker *et al.* (1979) on lung autotransplantations which were often unsuccessful when performed at the level of the proximal part of the main stem bronchus, indicating therefore that this segment of the airways is uniquely perfused by the systemic circulation.

The intraparenchymal bronchial receptors were stimulated by the R. H. injections of veratridine in a time interval shorter than that for their excitation through the systemic circulation (Fig. 2 and Table I). This supports a preferential perfusion—at least in our experimental conditions—of these airways, up to the second generation, through the pulmonary circulation.

The time interval between injection of veratridine into the left heart and the onset of stimulation of these bronchial receptors was very variable, but always much longer than after R. H. injection. Furthermore, the increase in receptor activity was, with one exception, less than with R. H. injection (Table I).

A possible explanation is that these endings can only be reached through the pulmonary circulation and therefore the delay for the onset of stimulation after L. H. injection would include left to right heart circulation time (6 sec; Spector, 1956) and a longer diffusion time (as compared to R. H. injection) for the reasons discussed for the tracheal receptors.

On the other hand Widdicombe (1954) showed that the stretch receptors in the intrapulmonary airways could be stimulated by veratridine directly injected into the bronchial circulation, though only after a considerable delay (about 30 sec.) Furthermore, anatomical studies have convincingly established that the bronchi are perfused by the systemic circulation (Miller, 1937; Cudkowicz and Armstrong, 1951; Nagaishi, 1972).

In any event it seems reasonable to assume that, at least in our experimental conditions, the patency of the bronchial circulation does not provide a " functional " access to the bronchial intrapulmonary receptors considered. They are instead preferentially accessible through the pulmonary circulation, even when located in larger airways. Therefore receptor localization based on the common assumption that an ending more accessible through the pulmonary than through the systemic circulation is located in very peripheral airways and, conversely, one reached more easily through the systemic circulation is located in larger airways, may be inaccurate.

Several chemical substances are either entirely or partially removed from the mixed venous blood by the metabolically active lung tissue and thus a gradient in their concentration is established between blood in the pulmonary circulation and in the systemic circulation.

Some of these substances (i.e. acetylcholine, prostaglandins E_1, E_2, F_{2a}, serotonin, bradykinin) are known to be active on one type or another of the pulmonary receptors (Sampson and Vidruk, 1978; Coleridge *et al.*, 1978) and therefore it is of considerable physiological interest to establish the preferential perfusion route for airways of different generations since, at least the endings having medullated fibers, are particularly concentrated in large airways.

Our results give grounds for the possibility that a significant proportion of airways receptors are affected by several active substances present in the mixed venous blood, but not in the systemic blood.

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