

# Computerized analysis of isometric tension studies provides important additional information about vasomotor activity

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## Abstract

Concentration-response curves of isometric tension studies on isolated blood vessels are obtained traditionally. Although parameters such as  $I_{\max}$ ,  $EC_{50}$  and  $pA_2$  may be readily calculated, this method does not provide information on the temporal profile of the responses or the actual nature of the reaction curves. Computerized data acquisition systems can be used to obtain average data that represent a new source of otherwise inaccessible information, since early and late responses may be observed separately in detail.

## Key words

- Vasoactivity
- Digitalization
- Substance P
- Acetylcholine
- VIP
- CGRP

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Isometric tension studies have traditionally employed mechanical multichannel recorders to measure changes in blood vessel tension under the influence of neurotransmitters, modulators, drugs and other vasoactive agents (1). Pharmacological data such as concentration-response curves may be obtained and values such as  $I_{\max}$ ,  $EC_{50}$  and  $pA_2$  readily calculated.

However, the application of computerized systems for data acquisition now extends the information available from such reactions. The ability to obtain accurate average data regarding the speed of a reaction and the nature of curve development permits the study of such reactions from a new point of view which can be illustrated with examples from our own work (2-4).

Circular artery segments (1 to 3 mm) are

mounted on two L-shaped steel holders (0.1 mm in diameter), one of the holders being connected to a Grass FT03C force-displacement transducer. The holders are immersed in 5 ml buffer solution through which a 5%  $CO_2/95\% O_2$  mixture is continuously bubbled for pH control. To record the arterial constriction forces, the transducers are linked to an analog-digital converter; in our case MacLab from Analog Digital Instruments (Milford, MA, USA), through a Transbridge TBM4 preamplifier (World Precision Instruments, Inc., New Haven, CT). The digitalized data are stored and analyzed with a MacLab Chart software in an Apple Macintosh computer. The system is adequate for detecting small changes in arterial constriction forces and the software automatically calculates several parameters such as maximum and

minimum values within a curve, eliminating errors which can occur when data are obtained manually from the recording paper.

Concentration-response curves are obtained by plotting the effect for each concentration of a vasoactive agent. Different substances may have the same maximum effect ( $I_{max}$ ), although the time course of the reactions may vary substantially. The concentration-response method does not detect such variability. Computerization introduces the possibility of studying reactions over time, and complements the information obtained in ordinary concentration-response studies. After an equilibration period, the substance under study is added once at a fixed concentration, usually that inducing the  $I_{max}$ . The precise time when the agent is added is accurately recorded by the computer, and the responses are continuously recorded thereafter (our system has been set for recording the tension every 6 s). The experiment can be repeated with as large a number of samples as desired and stored on a worksheet, after which an average digitalized curve representing the vasoactivity over time is easily obtained. This can then be reported, for instance, as percent relaxation of a  $PGF_{2\alpha}$ -induced pre-contraction if relaxing factors

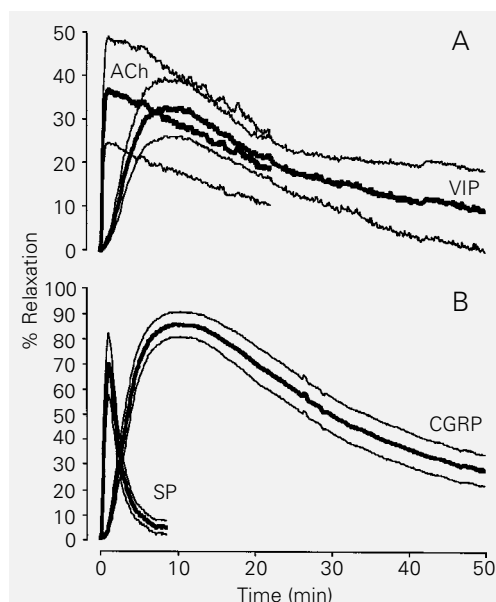
are to be studied, as in the examples here. Various parameters are readily calculated, such as  $I_{max}$ , the time necessary to reach  $I_{max}$  ( $t_{I_{max}}$ ), the tension present after the first 60 s or any other period of time, and the time necessary for 50% recovery ( $R_{50}$ ).

In the isolated porcine ophthalmic artery model, the endothelium-dependent relaxing agents substance P (SP) and acetylcholine (ACh) have a similar  $t_{I_{max}}$  of about 1 min, although the  $R_{50}$  is much shorter for SP (Figure 1). In contrast, vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP), which are endothelium-independent vasodilators, induce responses with similar time courses that are slow in onset and recovery (similar  $t_{I_{max}}$  and  $R_{50}$ ) but with different potencies (CGRP is more potent than VIP). The  $t_{I_{max}}$  for both CGRP and VIP is about 10 min. Similarly, ACh and VIP have a similar  $I_{max}$ , though  $t_{I_{max}}$  and  $R_{50}$  are quite different. The same is true for SP and CGRP. ACh and VIP co-exist in some parasympathetic fibers, while SP and CGRP co-exist in some sensory fibers (5). It is possible that SP is released when rapid vasodilatation is needed, whereas CGRP is preferentially released for slower, longer-lasting dilations.

Another advantage of the time course experiments is the ability to study the early phase of the vascular reactions which may be of particular importance under physiological conditions. In the isolated porcine ophthalmic artery, the vasodilatation observed one minute after the simultaneous addition of CGRP and SP was much greater than the sum of the individual SP- and CGRP-induced relaxations, suggesting a vasodilating synergism (3). Ordinary concentration-response curves performed in this artery by our group could not show any synergism between SP and CGRP.

The time course of reactions in this kind of model system will be characteristic for each type of substance according to its mechanism of action. It remains to be determined

Figure 1 - Average time curves for the reaction of 1  $\mu$ M acetylcholine (ACh, N = 6) and 10 nM vasoactive intestinal peptide (VIP, N = 8) (A), and 10 nM substance P (SP, N = 7) and 10 nM calcitonin gene-related peptide (CGRP, N = 8) (B) in isolated porcine ophthalmic artery. The relaxation curves obtained are reported as percentage of the  $PGF_{2\alpha}$ -induced pre-contraction. The bold lines represent the mean reaction + SEM (thin lines).



whether the reaction profile of a given vasoactive factor will indicate the activation of a particular cellular signalling system. Changes in tonus in isolated artery segments studied

from this angle seem likely to provide useful additional information about the actions of vasoactive factors and other substances modulating their activity.

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