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Review

Vane's blood-bathed organ technique adapted to examine the endothelial effects of cardiovascular drugs *in vivo*

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Abstract:
This study describes a modification of Vane's blood-bathed organ technique (BBOT). This new technique consisted of replacing the cascade of contractile smooth muscle organs within the traditional BBOT by a single collagen strip cut from a rabbit's hind leg tendon. Utilizing the extracorporeal circulation of an anesthetized heparinized mongrel cat or Wistar rat, arterial blood was dripped (1–3 ml min^{-1}) over a collagen strip. This resulted in a gain in weight of the strip, which was due to the deposition of platelet aggregates and a few blood cells trapped over the strip. Arterial blood that had been used for the superfusion was pumped back into the animal's venous system. However, when this technique is adapted to human volunteers, the superfusing blood should be discarded. In animal experiments, intravenous injections of a variety of classic fibrinolytic agents (e.g., streptokinase) promoted the formation of platelet thrombi. Nitric oxide donors (e.g., SIN-1) at non-hypotensive doses hardly affected the mass of platelet thrombi deposited over the collagen strip, whereas endogenous prostacyclin (e.g., released from vascular endothelium by bradykinin) or exogenous prostacyclin and its stable analogues (e.g., iloprost) dissipated platelet thrombi as measured by a loss in the weight of the blood superfused collagen strip. This model allowed us to assay numerous drugs for their releasing properties of endogenous prostacyclin from vascular endothelium. These drugs included lipophilic angiotensin converting enzyme inhibitors (ACE-Is), which act *in vivo* as bradykinin potentiating factors (BPF). Other PGI_2-releasers included statins (*e.g.*, atorvastatin and simvastatin), thienopyridines (e.g., ticlopidine and clopidogrel), a number of thromboxane synthase inhibitors, flavonoids, bradykinin itself, cholinergic M receptor agonists and nicotinic acid derivatives. The thrombolytic actions of lipophilic ACE-Is (e.g., quinapril and perindopril) were prevented by pretreatment with either bradykinin B_2 receptor antagonists (e.g., icatibant) or with endothelial COX-2 inhibitors (e.g., rofecoxib, celecoxib and high dose aspirin). The inhibition of endothelial nitric oxide synthetase (eNOS) by L-NAME hardly blunted the thrombolytic response to ACE-Is. Hence, it can be concluded that many recognized cardiovascular drugs apart from their known basic mechanisms of action, may also behave as releasers of endogenous endothelial prostacyclin. Furthermore, in many instances, this effect may be the primary mechanism of their therapeutic efficacy.

Key words:
endothelium, blood platelets, bioassay for prostacyclin releasers, ACE-I, BPF, bradykinin, statins, COX-2, flavonoids, thienopyridines, TXS-I, nicotinates, coxibs, aspirin

Abbreviations: ACE-I – angiotensin converting enzyme inhibitors, BPF – bradykinin potentiating factors, COX-2 – cyclooxygenase-2, Coxibs – COX-2 inhibitors, PGG_2, PGH_2 – prostaglandin endoperoxides, PGI_2 – prostacyclin, TXA_2 – thromboxane A_2, TXS-I – thromboxane synthase inhibitors

Introduction

Early days of the bioassay technique

The first *in vitro* bioassay system was invented by Rudolf Magnus during his stay at Heidelberg University in 1904 [28]. Magnus and his co-workers demonstrated that a piece of intestine isolated from a laboratory animal could survive *in vitro* when immersed in a bath filled with artificial physiological buffer. Moreover, this tissue was capable of contractions or relaxations in response to natural biological mediators and a number of various chemical stimuli. These dynamic responses of the isolated intestine could then be recorded by a system of levers. Subsequently, many outstanding pharmacologists [7, 35, 36] have constructed modified experimental bath units, altered the composition of the physiological buffer and have broadened the scope of smooth muscle organs that have been used for bioassay.

Vane's superfusion cascade

However, a fundamental modification of the classic bioassay system consisted of the replacement of the conventionally used *"immersing"* approach by a new *"superfusing"* approach [37]. This modification enabled Sir John Vane to arrange the detector organs in a cascade, which allowed the physiological fluid (e.g., Tyrode's buffer) or blood next to the first detector organ (e.g., a rabbit aortic strip) to be dripped down to the next one (e.g., rat stomach strip) that could then be dripped down to the next one (e.g., chick rectum), etc. This approach has been utilized to assay up to six different smooth muscle detectors, which were organized in the famously versatile "Vane's Bioassay Cascade." The careful choice of these "detector organs" resulted in characteristic patterns of contractions and relaxations along the cascade in response to various biologically active substances. These "biological fingerprints" allow experimenters to differentiate between known active substances, and they can be used to discover new biological mediators, such as in the case of prostacyclin [9]. At the top of the Vane cascade, there is usually mounted "a generator" that is a perfused isolated organ or a chamber filled with organ slices or cultured cells capable of producing and releasing biologically active substances in response to adequate stimuli. Subsequently, the presence of these biologically active substances manifests as a characteristic pattern of contractions and relaxations along the bioassay cascade.

For instance, the famous study of Priscilla Piper and John Vane [31] used the antigen-challenged lungs of a sensitized guinea pig as the generator, while a Krebs-Henseleit buffer was used as the perfusion fluid. In addition to histamine, prostaglandins and the slow reacting substance of anaphylaxis (SRS-A, which was later identified as leukotriene), the challenged lungs also released an uncharacterized "rabbit aorta contracting substance" (RCS). In similar experiments with greyhound spleen, we were able to identify RCS as a mixture of unstable prostaglandin precursors [17, 18] with an admixture of an even less stable compounds that decomposed to biologically inactive products in a matter of seconds [18]. Currently, we know that RCS is a mixture of PGG_2, PGH_2 and TXA_2 [9, 35].

Vane's blood-bathed organ technique (BBOT)

A breakthrough in the bioassay methodology occurred when John Vane decided to superfuse his cascade of detectors with blood withdrawn from and returned to the circulation of anesthetized, heparinized dogs [37]. Consequently, his famous blood-bathed organ technique (BBOT) was introduced to a host of physiological and pharmacological laboratories. Furthermore, this technique lead to many basic discoveries that included the effect of circulating blood volume on the endogenous generation of angiotensin [22], the consequences of acute coronary occlusion on the release of endogenous catecholamines [3, 21], differences in the removal routes from circulation of exogenous noradrenaline and isoprenaline [16] and mechanisms underlying the release and disappearance of histamine from circulation [6].

Materials and Methods

Our modification of BBOT

Here, we present a summary of our modifications [1, 2, 8–15, 23–25, 27, 30, 33, 34] of Vane's BBOT [37]. These modifications enabled us to discover the endothelial prostacyclin-mediated mechanism of action for several cardiovascular drugs. In essence, we replaced

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