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Basic research models for the study of underlying mechanisms of electrical neuromodulation and ischemic heart-brain interactions

ABSTRACT

The study of mechanisms of action underlying the use of electrical neuromodulation for angina and myocardial ischemia may illuminate heart-brain interactions that influence these conditions. To investigate these mechanisms of action, we initiated a neurocardiology program in the 1990s. This review discusses the experimental models we have studied to unravel the heart-brain interactions involved in the use of electrical neuromodulation for ischemic disease.

RATIONALE

In the industrialized world, average life expectancy has nearly doubled since the 19th century. One of the consequences of this increase in life span is that the sequelae of diseases also have increased. For coronary artery disease (CAD), one of the most prevalent diseases in the western world, this has resulted in an amplification of the number of patients suffering from heart failure, arrhythmias, and refractory angina. Much progress has recently been made in nonpharmacologic therapies for these deleterious consequences of CAD, such as cardiac resynchronization for heart failure, implantable defibrillators for ventricular arrhythmias, and electrical neuromodulation by means of spinal cord stimulation for chronic angina that is refractory to conventional strategies.

For patients suffering from severe angina secondary to end-stage CAD who have no other options to alleviate their complaints, electrical neuromodulation may be the preferred adjunctive treatment.[1] Although spinal cord stimulation is still not approved by the US Food and Drug Administration for treatment of refractory angina, it is is accepted in the American College of Cardiology/American Heart Association guidelines for chronic stable angina, with a class II indication, and is frequently used for this indication in Europe.[2]

However, to understand underlying mechanisms of therapies such as electrical neuromodulation—executed through either transcutaneous electrical nerve stimulation or spinal cord stimulation—for angina pectoris and to improve the effect and safety of these therapies, clinical questions concerning neuromodulation must be evaluated in experimental models. The outcomes of these preclinical experimental studies subsequently need to be assessed in humans.

Although therapeutic improvements from implantable devices would not have been possible without experimental work, any experimentation must be avoided if it is not approved by the relevant ethics committee(s) or is not conducted in keeping with standard guidelines. For this reason it is sometimes more feasible, when appropriate, to make use of simulation models—for instance, to study regularization of atrial fibrillation by means of a device.[3,4]

So, on the one hand it is challenging to use electrical neuromodulation as a tool to study heart-brain interactions in general; on the other hand, electrical neuromodulation may be used to study its own underlying mechanisms of action, more specifically on characteristics of angina and myocardial ischemia. To investigate these mechanisms of action of electrical neuromodulation, we initiated a neurocardiology program in the 1990s **(Figure 1)**. This article will discuss the experimental models we have studied to unravel the heart-brain interactions involved. We studied electrical neuromodulation both in patients and in experimental animals. However, the lack of knowledge about fundamental aspects of cardiovascular regulating circuitry and cardiac pain, as well as the lack of an animal model for angina pectoris, is the background for the various projects we have conducted concerning heart-brain interactions.

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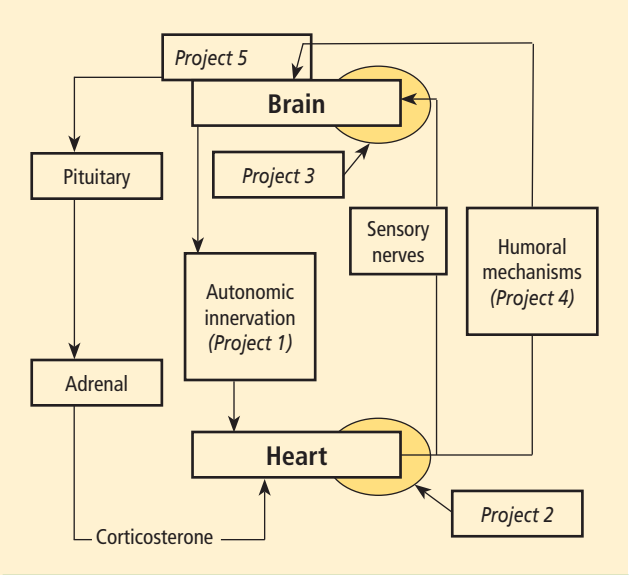

FIGURE 1. Our preclinical neurocardiology research program. Several experimental approaches, ranging from neuroanatomy to molecular biological studies of cardiac nociceptor mRNA expression, have been employed to unravel mechanisms of heart-brain interaction and electrical neuromodulation. For explanations of project numbers, see the text.

PROJECT 1: EMOTIONS AND MYOCARDIAL ISCHEMIA

In 1772, Heberden described to physicians in England the clinical symptoms of exercise-induced chest discomfort, with its emotional component and vaguely distributed projection on the chest, as follows: "The seat of it, and sense of strangling and anxiety with which it is attended, may make it not improperly be called angina pectoris."[5] Since then, it has been demonstrated repeatedly that strong emotional distress frequently precedes or is associated with complaints of pain in the chest. Further, emotional suffering has been associated with increased mortality in patients with CAD. We and others, unfortunately, were confronted with very limited knowledge of the precise locations of the origin of emotions in the limbic structures of the forebrain. Even less was known about the relationship of these brain structures and the heart, owing to technical limitations in the field of neuroanatomical tract tracing, among other reasons. As a result, the nervous pathways from the heart, through which signals are propagated to the brain in order to activate emotional components, were not accurately identified. We therefore initiated Project 1 to study, in a rat model, neuroanatomical characterization of the neuronal circuitry controlling cardiac activity, specifically during cardiac distress.

In the area of identifying efferent neural pathways from the heart, we were the first to publish an experimental setup making use of a neurotropic herpesvirus from the Bartha strain of the pseudorabies virus (PRV).[6] Following injections of PRV into the left and right myocardium or into atrial tissue, PRV infects the neurons that innervate the injection site and is then transported in the neural network, where the virus may cross at least four synapses. This transneuronal retrograde viral pathway labeling method with PRV provided us the opportunity to study cardiovascular controlling networks. The distribution of the PRV-infected cells was studied immunocytochemically after survival times of 3 to 6 days. Right ventricular infection showed labeling in the same nuclei as left ventricular labeling, but the number of PRV-positive cells was always higher and the localization of PRV within the nuclei differed. These obvious signs for differentiation within the nuclei suggest differential neuronal pathways to various parts of the heart.

Following injection of PRV at different cardiac sites, differences in density and localization of PRV-positive cells were found predominantly in higher-order neurons that are known to be involved in cardiac control. Transection of the spinal cord at Th1, performed to reveal selectively the parasympathetic neuronal networks, reduced the number of labeled cells, specifically in the periaqueductal gray matter. Virus-labeled sympathetic preganglionic cells were found in the Th1–Th7 thoracic intermediolateral cell groups, with some additional infections at Th8–Th11 after inoculations of the ventricular myocardium. The rostral parts of the insular cortex appeared to be linked selectively to sympathetic innervation of the heart.[6]

From the experiments we hypothesized that, according to the type of lesion, the pattern of cardiac innervation may account for a specific malfunctioning. Subsequently, the subendocardial clustered parasympathetic nerves make these nerves more vulnerable for myocardial damage than the superficial spread of sympathetic nerves. In this respect, the identification of three preganglionic parasympathetic nuclei in cardiac control—ie, the dorsal motor nucleus of the vagus (20% labeling), the nucleus ambiguus, and the periambiguus—constituted the most striking findings.

PROJECTS 2 AND 3: CARDIAC NOCICEPTOR ACTIVATION

The cortical structures and their related output pathways also serve as effector systems for initiation of autonomic and behavioral responses by forebrain neuronal networks that make us aware of cardiac pain. However, these cortical and subcortical structures

involved in cardiac pain perception were more or less terra incognita. In addition, we studied fundamental aspects of cardiac nociceptor activation (Project 2) and transduction of cardiac pain (Project 3). Unfortunately, there was no experimental animal model for angina pectoris. The aim of these projects was to obtain, both in patients and in animals, knowledge about cardiac nociceptor activation mechanisms, the transmission and perception of cardiac pain, and behavioral and autonomic responses.

To enable the study of mechanisms of neurostimulation during episodes of acute cardiac pain, we worked out an animal model for angina pectoris. For that reason we experimented with models in which we created an acute myocardial infarction. We had to reject this model since surgery and, more importantly, anesthesia interfered with the patterns of cerebral expression of immediate early genes (c-fos, c-jun) triggered by cardiac pain and/or neurostimulation. However, a spinoff from this project was the observation that cardiac tissue damage causes a reproducible and selective cerebral endothelial leakage of immunoglobulin G (IgG) molecules. Follow-up experiments showed that proinflammatory cytokines, which are released into the circulation after cardiac tissue damage, can generate the same pattern of blood-brain barrier dysfunction[7] (see Project 4).

We then experimented with infusions of capsaicin into the pericardial space of unrestrained and unanesthetized rats to induce acute cardiac pain. This model appeared to be very promising and allows visualization of the behavioral and autonomic responses to cardiac pain. Cerebral c-fos expression patterns, a marker for structures involved in cardiac pain transmission and perception, were studied and validated with positron emission tomography (PET) imaging in patients.[8]

Project 2: Nociception of cardiac pain in patients

To study relationships between neurotransmitters and other molecules that contribute to pain and psychological variables, we studied cardiac tissues obtained from 22 patients with angina during coronary artery bypass graft surgery (CABG). Cardiac nociceptor activation mechanisms were investigated in heart biopsies from these 22 CABG patients; reverse transcriptase polymerase chain reaction analysis (RT-PCR) was conducted for adenosine and bradykinin receptor mRNA.[9,10]

An age-related decrease was observed in the adenosine A1 mRNA density but not in the bradykinin receptor mRNA levels. The adenosine A1 receptor density also correlated with pain characteristics reported in a questionnaire. Making use of semiquantitative RT-PCR, cardiac tissue substrates were assessed to determine the expression of adenosine A1 and bradykinin B1/2 receptor mRNA densities. The outcomes were associated with the quality of pain, age, gender, medication, and duration of disease.[9,10]

For evaluation of pain characteristics, we used questionnaires and objective pain scores. We found that qualitative age-related alterations in angina perception correlated with the development of the more "strangling" component of angina at older age. This observation may be explained, in part, by a reduction in adenosine A1 receptor mRNA expression in the heart, since bradykinin B1/2 receptor densities remain the same.[9,10]

Project 3: Nociception of cardiac pain in unrestrained rats

Having identified neural pathways, we studied neurons that were activated during electrical neuromodulation.[11] In search of a putative mechanism of action of electrical neuromodulation, we hypothesized that neuromodulation affects processing of nociceptive information within the central nervous system (CNS). To characterize neural activity we used expression of both the immediate early gene c-fos and the "late gene" or stress protein known as heat shock protein 72 (HSP72). c-fos was used to identify structures in the CNS affected by spinal cord stimulation. HSP72 was applied to ascertain whether spinal cord stimulation might operate as a stressor.[12]

Animal experiments were conducted on unrestrained unanesthetized rats implanted with a permanent catheter in the pericardial space; acute cardiac pain was triggered in this space using capsaicin as the algogenic substance.[13] The autonomic cardiovascular responses were recorded with implantable telemetric devices. Behavioral responses were recorded on videotapes taken from the same animals in which the involved cerebral structures were characterized by analyzing cerebral immediate early gene expression. Quantification of data makes it possible to study the effects of electrical neuromodulation and analgesic drugs on perception of cardiac pain. To apply electrical neuromodulation, two electrodes were positioned and sutured epidurally at the spinal cord of the rats. One electrode was fixated at spinal nerve C7 and the other at T2. Furthermore, we studied the effect of spinal cord stimulation on behavior. Three hours after stimulation, the rats were sacrificed and their brains and spinal cords were removed.

The treated group showed regional increased c-fos expression in a select group of regions of the limbic

system—periaqueductal gray, paraventricular hypothalamic nucleus, paraventricular thalamic nucleus, central amygdala, agranular and dysgranular insular cortex, (peri)ambiguus, nucleus tractus solitarius, and spinal cord—involved in the processing of pain and cardiovascular regulation, among other functions. Moreover, in both treated rats and controls, HSP72 expression was found in the endothelium of the enthorhinal cortex, the amygdala, and the ventral hypothalamus, but not in the neurons. The treated animals were significantly more alert and active than were the controls.

Thus, the rat model we developed appears to be suitable for studying potential mechanisms through which neuromodulation may act. Moreover, neuromodulation affects c-fos expression in specific parts of the brain known to be involved in regulation of pain and emotions. HSP72 expression is limited to the endothelium of certain parts of the CNS, and thus physical stress effects were excluded as a potential mechanism of neuromodulation. Finally, our experimental model identified regions corresponding with regional cerebral blood flow changes during neurostimulation in patients.[8]

PROJECT 4: BIDIRECTIONAL HUMORAL AND NERVOUS HEART-BRAIN INTERACTIONS

With respect to the emotional component of angina, we thought to study alternative pathways of communication between the heart and the brain. This idea occurred as a consequence of observations that many patients who suffer serious cardiac events, such as CABG or myocardial infarction, are confronted with a period of emotional problems following these events. So, from our experimental projects, the question became relevant as to whether emotional alterations in behavior following a cardiac life event may be executed by a humoral pathway from the heart to the brain, since, vice versa, the brain controls the heart through both nervous and humoral pathways. In other words, is it feasible that both humoral and neural pathways are involved, bidirectionally, in interactions between the brain and the heart?

Cardiac disease, proinflammatory cytokines, and blood-brain barrier damage

Cardiac ischemia, the underlying cause of cardiac pain in angina pectoris, triggers a cascade of events that release numerous substances in the myocardium and circulation, all of which are potential candidates for nociceptor activation and initiation of behavioral and autonomic responses to cardiac pain. Some of the substances that are released into the circulation may play a role in the humoral communication between heart and brain, but when released chronically, these substances may induce neuropathological modifications. Anxiety disorders and depression are cerebral disorders that are frequently comorbid with ischemic heart diseases. The latter are attributed to noncoping behavior, but our own experiments (as part of the program) showed that immune activation after tissue damage in the heart generates regional blood-brain barrier damage (Project 4) that could be an underlying organic basis for comorbid neuropsychiatric disorders. The incentive for this project in general was the observation that myocardial infarction is accompanied by behavioral and neuronal abnormalities.

In this project we established whether release of proinflammatory cytokines after tissue damage in the heart is a possible inducer of comorbid neuropsychiatric diseases.

As a model for immune activation, we studied the effects of intravenous injections of the proinflammatory recombinant tumor necrosis factor–alpha (TNF-α) on cerebral endothelial leakage, induction of neuronal damage, and motor and cognitive function in rats. Determinants of selectivity of blood-brain barrier damage were assessed with a molecular biological approach in which we studied regional differences of TNF-α–induced expression in the cerebral endothelial cells of the immediate early gene c-fos and proteins involved in leukocyte docking (intercellular adhesion molecules [ICAMs]) and TNF-α receptors.

To examine the mechanisms by which this interaction occurs, we induced myocardial infarction in a group of rats and then performed immunohistochemistry of the brain. This experiment revealed regional serum protein extravasation, pointing to leakage of the blood-brain barrier. This process occurred in certain cortical, subcortical, and hindbrain areas in discrete patches. The leakage was colocalized with expression of the immune activation marker ICAM-1. To assess the involvement of the immune system in the effects shown, a second group of rats was injected with TNF-α, as the major proinflammatory cytokine. This procedure rendered the same results. It was concluded that myocardial infarction may interfere with the integrity of the blood-brain barrier and possibly with brain functioning through activation of the immune system. The relevance for pathophysiological processes may provide a substrate for further research in unraveling the emotional consequences of serious cardiac events.

In the state of immune activation that follows myocardial ischemic events, various cytokines are released from the myocardium into the plasma. These cyto-

kines potentiate the cytotoxicity of TNF-α. In the next experiment we were able to demonstrate that intravenous injection of TNF-α induces a selective and regional neural IgG and endothelial ICAM-1 immunoreactivity. The expression of TNF-α–induced changes in the brain suggests that TNF-α is capable of inducing blood-brain barrier dysfunction. It is hypothesized that through dysfunction of the blood-brain barrier, the released cytokines bind to specific cognitive centers in the brain and thus may lead to emotional disturbances following cardiac events.[14]

Having identified some specific centers involved in cardiovascular control, we further studied the effects of electrical and chemical stimulation of a specific brain center on the heart.

PROJECT 5: EFFECT OF BRAIN STIMULATION ON CORONARY FLOW

From a clinical PET study performed in patients with end-stage CAD during active spinal cord stimulation therapy, as well as from our PRV experiments and the literature, we concluded that the periaqueductal gray plays a central role in the regulation of different cardiovascular responses and in the integration of motor output from the limbic system.[6,7] Subsequently, the periaqueductal gray has been thought to be one of the pivotal cerebral centers involved in executing electrical neuromodulation effects.

We investigated the function of the periaqueductal gray in regulation of the coronary flow of the heart. Depending on the stimulation site, electrical stimulation in the periaqueductal gray resulted in increases and decreases in coronary flow and conductance. These effects were organized topographically. The sites producing increases in coronary flow and conductance were found in both the dorsolateral and the ventrolateral periaqueductal gray. The sites producing decreases were restricted mainly to the ventrolateral portion. Similar topographic distributions were observed for the sites producing changes in carotid conductance and heart rate, but not for those producing changes in blood pressure and carotid flow. It is hypothesized that the topographic distribution of coronary vasoconstrictive and vasodilatory responses from the periaqueductal gray may enable optimal adjustments of the coronary perfusion. These optimal adjustments can then accommodate variations in myocardial oxygen demands accompanying different behavioral modes.

CONCLUSION

From all our experiments, mainly performed in rats (but sometimes also in a cat model due to the existence of a stereotactic brain atlas for the cat), we have learned about heart-brain communication through the use of electrical neuromodulation. In the last decade we have further studied heart-brain interactions in the International Working Group on Neurocardiology (IWGN), making use of canine and rabbit models. The main focus of the IWGN is on neural hierarchy in cardiac control. These projects are discussed by one of us (R.D.F.) elsewhere in these proceedings. In brief, the importance for the heart of the intracardiac neuron system and controlling centers at the C1 spinal level,[15–17] in conjunction with the induction of myocardial ischemia, will be highlighted. For a more extensive overview of recent work performed by the IWGN, see the reviews by Foreman et al[18] and Wu et al.[19]

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