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Collaborating with Alexander Scriabine and the Miles Institute for Preclinical Pharmacology

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ABSTRACT

This article represents a timely opportunity to express my affection, admiration and gratitude to Professor David Triggle. David was my Ph.D. advisor as well as a key consultant in the 1980s and early 1990s for research programs at Miles Institute for Preclinical Pharmacology in West Haven, CT, the U.S. research operation of Bayer AG, in the areas of Ca^{2+} and K^+ channel ligands. The binding methodology developed in his laboratory was used to search for an endogenous ligand for L-type Ca^{2+} channels. We did not find the substance that we were searching for, a genetically-determined, competitive inhibitor for the 1,4-dihydropyridine binding site, but instead isolated the endogenous ligand for the brain's own marijuana, anandamide. Devane, Mechoulam and coworkers first discovered that this compound was the endogenous ligand for delta-9-tetrahydrocannabinol, the active substance in cannabis. The endogenous endocannabinoid system is now the target of many exciting new approaches to drug discovery.

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1. Introduction

In 1979, Bayer AG bought Miles Laboratories in Connecticut and recruited the accomplished pharmacologist and drug hunter, Alexander Scriabine, M.D. from Wyeth to establish a new institute to carry out and promote research on Bayer drugs in development. Alex was responsible for directing all preclinical pharmacological research and development of compounds selected by Bayer, including preclinical experiments that were performed by outside contractors and universities.

Initially Alex rented space from Yale University in New Haven to facilitate the rapid start of these programs. The Miles Institute for Preclinical Pharmacology (Miles Institute) was first established in the Osborn Memorial Laboratories at Yale University before moving to new laboratories in West Haven, which eventually became the Bayer Research Center. David Triggle was one of the first consultants to be engaged by Alex (Fig. 1). This was an insightful choice as one of the Institute's major projects was a search for an endogenous ligand for the 1,4-dihydropyridine receptor of the L-type Ca^{2+} channel. We did not find an endogenous

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Fig. 1. Dr. David Triggle and Dr. Alexander Scriabine at a reception at Bayer AG.

competitive inhibitor for the 1,4-dihydropyridine binding site, but instead isolated anandamide, the brain's own marijuana [1]. The former Bayer Research Center in Orange and West Haven CT is now part of Yale University.

2. Ca^{2+} channel ligands

David Triggle had already begun nitrendipine binding in his laboratories at SUNY-Buffalo in 1981 when Alexander Scriabine decided to use this approach to characterize binding sites for Bayer drugs. George Sarmiento from the Miles Institute visited David's laboratories to learn these techniques. The results of the initial studies on the high affinity binding of nitrendipine and nimodipine to smooth and cardiac muscle membranes were published jointly, which was the beginning of several years of productive collaboration. Nitrendipine and nimodipine binding to smooth and cardiac muscle membranes showed a similar high affinity and specificity [2–5]. The good agreement between the characteristics of binding and pharmacology indicated that the binding site was mediating the pharmacological properties of the L-type Ca^{2+} channel inhibitors. The first and most extensive characterization of the nitrendipine binding site in smooth muscle was done in David's laboratory [2,4]. In this detailed study using a microsomal preparation of guinea-pig ileal membranes, pharmacological relevance, structure-activity correlations, stereoselectivity, and modulation by other classes of Ca^{2+} antagonists were demonstrated. An excellent correlation between the absolute potency of a variety of nifedipine analogs for inhibition of nitrendipine binding and for inhibition of ileal contractions induced by K^+ depolarizing solution was found, see Fig. 4, in [5] this issue. Thus, the most important criterion for establishing the pharmacological relevance of the binding site was satisfied. A dozen early studies reported a similar high affinity binding site in membranes from a variety of smooth muscles (reviewed in 6,7). Known Ca^{2+}antagonists inhibit binding of 1,4-dihydropyridines, but a large number of other compounds tested were inactive [3–6].

The high affinity 1,4-dihydropyridine binding site in purified sarcolemma from canine cardiac ventricle was found to be similar to that in bovine aortic smooth muscle, but the density of sites in ventricle was 10-times larger [8]. The discrepancy between the insensitivity of cardiac cells to these drugs and the high binding affinity was attributed to the presence of a high affinity inactivated state of the channel in isolated cardiac membranes [7,9]. The effects of metal cations and calmodulin antagonists on nitrendipine binding to smooth and cardiac membranes suggested that calmodulin antagonists bind to a protein associated with the nitrendipine binding site that has a hydrophobic domain similar to that exposed on calmodulin by Ca^{2+}, but that this protein is not calmodulin itself [10].

A variety of studies on the effects of a channel agonist on nitrendipine binding were made possible by the availability of the BAY K 8644 [11,12]. The high affinity binding site for BAY K 8644 was affected by some of the same factors that modify antagonist binding, including the non-dihydropyridine antagonists, indicating a common site of action [13,14]. These studies suggest that the organic Ca^{2+}antagonists do not physically block the Ca^{2+} channel, and they support the hypothesis that the high affinity binding of nitrendipine in isolated cardiac membranes was to Ca^{2+} channels that were inactivated or were otherwise unavailable for opening [14]. The many structural features of activator and antagonist ligands that may contribute to their ability to stabilize either the open or closed state of the channel have been analyzed [15,16]. More detailed examination of the action of the (-)-activator isomer of BAY K 8644 showed that it could be either an activator or an antagonist, depending on the membrane potential. Thus, the effect of 1,4-dihydropyridines depends not only on their chemical and configurational properties, but also on the channel subtype and state [15].

A summary of 150 binding studies done between 1981 and 1987 shows that membranes from different tissues generally exhibit the same high affinity for nitrendipine (about 0.1 nM), except for skeletal muscle, which has about a 10-fold lower affinity [16]. In addition, the affinities of the binding site in several tissue types show a similar temperature dependence, consistent with the identity or near identity of those sites [17]. Vascular smooth muscle sarcolemma, the major clinical site of action of nifedipine and related drugs, appears to have the lowest density of sites, less than 1 pmol/mg, whereas skeletal muscle t-tubules have the highest density, greater than 80 pmol/mg [17,18].

The insensitivity of neuronal tissues and skeletal muscle to the 1,4-dihydropyridines is consistent with their current classification as separate subtypes of voltage-dependent Ca^{2+} channels. The skeletal muscle subtype of L-type channel is classified as $Ca_v1.1$, the cardiac, smooth muscle, and endocrine, $Ca_v1.2$ and one neuronal type is $Ca_v1.3$ [19,20].

3. K^+ channel ligands

K^+ channel activators, like L-type Ca^{2+} channel blockers, have potent vasodilating and smooth muscle relaxing properties, and therefore are of interest in drug discovery for the treatment of several disorders, including hypertension, asthma and urinary incontinence [21]. Collaborative studies between David's laboratory and Miles Institute characterized the binding of [^{3}H]glyburide to ATP-dependent K^+ channels in muscle and neuronal preparations. The results suggested that there may be multiple types of ATP-dependent K^+ channels [22]. Currently, the classification of these inward rectifying channels includes two subtypes of channels sensitive to sulfonylureas and K^+ channel activators [23]. Following these initial studies with David and Murali Gopalakrishnan, Miles Institute described for the first time the high affinity binding of a K^+ channel agonist to intact cells [24]. This high affinity binding to insulinoma cells was inhibited by a series of BAY X 9228 analogs with an activity sequence correlating well with that for producing glyburide reversible relaxation of partially depolarized rat aorta. Assays using new K^+ channel openers are