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Electrophysiological Assay and Characterization of Central Adrenoceptors: Techniques and Neuropharmacology

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MARWAHA, J. AND A. L. CURTIS. *Electrophysiological assay and characterization of central adrenoceptors: Techniques and neuropharmacology.* PHARMACOL BIOCHEM BEHAV 22(5) 875–880, 1985.—Several single unit electrophysiological studies that have investigated central adrenoceptors are reviewed. The techniques and paradigms employed to electrophysiologically assay such adrenoceptors are discussed. Several regions of the brain, e.g., the nucleus locus coeruleus, the dorsal raphe nucleus, the lateral geniculate nucleus, and the cerebellar Purkinje neurons, are examined in detail, with reference to the nature of the adrenoceptor(s) located on these neurons. From the studies reviewed, it can be concluded that single unit electrophysiological recordings provide a valuable and powerful assay for adrenoceptors. Modification of this technique to study adrenoceptors from awake (behaving) or chronically treated animals is likely to result in significant advances in our understanding of mechanisms contributing to neuroreceptor plasticity.

Adrenoceptors Electrophysiology Single unit studies Receptor plasticity Locus coeruleus
Purkinje cells Microiontophoresis Chronic unit recording Alpha adrenoceptors Beta adrenoceptors

NEURORECEPTORS are regarded as dynamic entities which provide an accessible clinical handle. In recent years, considerable research effort has been expended in studying neuroreceptor properties. Several techniques, e.g., ligand binding, metabolite measurements, *in vitro* tissues, autoradiography, electrophysiology, behavioral techniques, neuroendocrine approaches, etc., have been utilized to study receptor properties. Some of these techniques measure part of the primary event, e.g., binding of agonist molecules to recognition sites of the receptor, whereas others measure non-primary events (parameters), e.g., inhibition of cell firing, metabolite measurements, contraction of a tissue, etc.

This chapter reviews some studies that have employed single unit electrophysiological techniques to characterize central adrenoceptors. Single unit recording is a relatively unobtrusive technique. It produces minimal perturbation of the biological system; it permits temporal correlations to be made between changes in neuronal physiology and those in the brain, and it permits a high resolution of morphological specificity, since changes can be traced to single cells at a specific location. The objective in such studies has been to assay the properties of a single population of receptors on single identified neurons.

Classification of adrenoceptors into two main types, alpha and beta, was originally proposed by Ahlquist [2]. Currently, four major groups of adrenoceptors (e.g., alpha-1, alpha-2, beta-1, and beta-2) are recognized centrally. With the availability of more specific pharmacological tools, additional subclassification of adrenoceptors is likely. Presynaptically located alpha-adrenoceptors have been identified as alpha-2 since they are preferentially activated by alpha-2 adrenoceptor selective agonists, and blocked by alpha-2 adrenoceptor selective antagonists. Presynaptic alpha-adrenoceptors have been proposed to mediate two functions; those located on the terminals determine the quantity of transmitter released per nerve impulse, while the receptors located at the neuronal soma and dendrites modulate the "firing frequency" transmitted to the axon hillock [27]. At least two subtypes of postsynaptically located alpha-adrenoceptors have been recognized; namely, alpha-1 and alpha-2. It has been proposed that alpha-1-adrenoceptors are located closer to the nerve terminal than the alpha-2-adrenoceptors [13]. Based on this suggestion, it follows that alpha-1-adrenoceptors would be activated maximally by sympathetic nerve stimulation whereas alpha-2 activation would be dependent on circulating catecholamines. In recent years, the concept has arisen that alpha-adrenoceptors should be classified as alpha-1 and alpha-2 according to their pharmacological responses irrespective of their anatomical location [5].

Both beta-1 and beta-2 adrenoceptors have been reported to exist centrally. It has been proposed that beta-1 adrenoceptors are those that are located on neurons, while beta-2 adrenoceptors are located extraneuronally [20]. How-

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TABLE 1

CURRENTLY AVAILABLE DRUGS† FOR ELECTROPHYSIOLOGICAL CHARACTERIZATION OF CENTRAL ADRENOCEPTOR SUBTYPES

Receptor	$Alpha_1$	$Alpha_2$	$Beta_1$	$Beta_2$
Agonists	*Phenylephrine	Clonidine	Dobutamine	Terbutaline
	St 587	α-methyl norepinephrine	Tazolol	Zinterol
	Methoxamine		Prenalterol	Salbutamol
		Guanfacine		
		UK-14,304		
		Guanabenz		
		BHT-920		
		BHT-933 (Azepexole)		
		ICI-160,270		
		*4-OH Clonidine		
		*Naphazoline		
		*Oxymetazoline		
		*ST-91		
Antagonists	Prazosin	Yohimbine	Metoprolol	Butoxamine
	Corynanthine	Rauwolscine	Practolol	H 35/25
	WB 4101	RX 781094 (Idazoxan)	Atenolol	
		Piperoxane		

*Polar or relatively polar compounds that do not easily cross the blood-brain barrier.
†This is *not* an exhaustive list, but rather a representative one.

ever, the electrophysiological characterization of β-receptor subtypes has *not* yet progressed to the same extent as that for central alpha receptors.

Single population(s) of receptors are studied by employing receptor subtype selective agonists and antagonists (Table 1). Initially, agents which did *not* discriminate between subtypes were employed. As more selective agents became available, these have gradually replaced the non-specific compounds. The availability of a variety of receptor subtype specific drugs has allowed greater resolution of receptor subtype populations and enabled investigators to begin research into the mechanisms of regulation and coupling of these receptor subtypes to their respective cellular effector systems. An important but frequently overlooked aspect regarding the choice of subtype specific agents is one of lipophilicity. Agents, such as the alpha-2-adrenoceptor agonist clonidine, are very lipophilic and, as a consequence, would be expected to rapidly cross cellular membranes [16]. Thus it may be difficult to determine the exact site of action of such compounds even after localized and restricted application. It is recommended that both lipophilic and nonlipophilic structural analogs of a compound be employed to reach valid conclusions about sites of drug action.

Identification of single neurons on which adrenoceptors are located has been achieved physiologically, pharmacologically, and anatomically. Some physiological criteria utilized to identify central neurons have included spike waveform and duration, discharge frequency, response to sensory or motor perturbations, and orthodromic/antidromic activation. Pharmacological identification has been ascertained on the basis of the neuroreceptors present on the neuron. If the identity of these neuroreceptors and their function has been previously established, then the response of the neuron to a well studied pharmacological agent can serve as an identifying feature. Anatomical identification has been achieved by dye ejection techniques, stereotaxic electrode placement, utilization of "key" landmarks, histochemical approaches (e.g., fluorescence) and autoradiography. The bias to date in most single unit electrophysiological studies has been to collect data largely from spontaneously firing neurons (as opposed to "silent" cells and/or "stimulated /driven" cells). This bias makes the rather unwarranted assumption that spontaneously firing neurons more accurately represent central neuronal physiology. This assumption is further weakened by studies which demonstrate that the spontaneous activity of most central neurons is governed by the anesthetic agent employed and the depth of anesthesia. As a consequence, the same neuronal system in a single species studied by different laboratories has at times provided conflicting data.

Electrophysiologically, receptor subtypes and their properties have been studied utilizing two major experimental paradigms: (a) *in situ* (intact animals); (b) *in vitro* (brain slices and cultured cells). Each paradigm has advantages and limitations and a comparison between these approaches is outlined in Table 2. Until recently, the "*in situ*" studies have predominantly employed anesthetized/paralyzed animals. Stability of recording has been a major reason for this approach. However, with the realization that anesthetic agents or animal immobilization may profoundly alter neuron behavior and responsiveness to pharmacological agents, awake, behaving animals have begun to be employed. Few of the currently available electrophysiological techniques can study properties of receptors located on nerve terminals.

These autoreceptors play an important role in modulating transmitter release. The assumption made with electrophysiological studies is that for a given receptor subtype the properties of terminal autoreceptors are identical to those in the soma-dendritic region. Additionally, for several years it was assumed that single neurons have single receptor populations and secrete single transmitters. The coexistence and association of diverse transmitters and receptors with single neurons has added another level of complexity in interpreting data from single unit studies. A question which as a consequence will need to be addressed is whether alteration(s) in plasticity of a given population of receptors affect the properties of adjacent (but different population) receptors on the same neuron. Much of the electrophysiological data generated to date has been from single neurons in a single region. It is widely accepted that the central nervous system consists of interconnected circuits and thus simultaneous recordings from multiple sites (neurons) in a connected circuit would yield additional useful information. A brief review of some reports that have employed single unit electrophysiological recording to study central adrenoceptors now follows.

In single cell recording studies, small systemic doses of clonidine inhibit the firing of neurons in the locus coeruleus [28]. The involvement of an alpha-2-adrenoceptor in these responses is supported by the additional finding that alpha-2 (piperoxane, yohimbine, and rauwolscine) but not alpha-1 (corynanthine, prazosin, and WB-4101) adrenoceptor antagonists can readily reverse the inhibition of locus coeruleus neurons produced by clonidine [7,14]. Additionally, alpha-2 adrenoceptor antagonists alone in small intravenous doses cause activation of locus coeruleus cell discharge [14]. Microiontophoretic application of norepinephrine, epinephrine, and clonidine inhibits locus coeruleus neurons. These inhibitory actions are antagonized by iontophoretic application of alpha-2-adrenoceptor antagonists but not beta-adrenoceptor or dopamine receptor antagonists [7]. Some of these latter studies are suggestive of a tonic inhibitory catecholaminergic input(s) to the locus coeruleus. These inputs could represent axon collaterals of the locus coeruleus neurons themselves or they reflect synapses of catecholaminergic neurons on locus coeruleus cells. Most adrenoceptors mediate alterations in ionic conductances which are specific to the type of adrenoceptor involved. Several studies have been conducted to determine the cellular mechanism(s) associated with central alpha-2-adrenoceptor activation. Systemic injection of clonidine elicits a hyperpolarization of noradrenergic locus coeruleus neurons and an inhibition of firing [1]. This hyperpolarization is accompanied by a reduction in input resistance. It has been suggested that an increase in potassium conductance contributes to both the hyperpolarization and decrease in input resistance [1]. Additional studies indicate that there exists a calcium activated potassium conductance in locus coeruleus neurons [3].

The dorsal lateral geniculate nucleus (LGNd) receives noradrenaline-containing afferents originating in the locus coeruleus. Electrical stimulation of the locus coeruleus activates LGNd neurons [23]. This effect is abolished when central noradrenergic stores are depleted. Norepinephrine applied by iontophoresis produces an increase in the firing rate of LGNd neurons an effect selectively blocked by alpha-1-adrenoceptor antagonists. In this system, norepinephrine activates neurons in the LGNd via a post-synaptic alpha-1-adrenergic receptor [22]. Alpha-1-adrenoceptor antagonists inhibit the response of LGNd neurons to alpha-1 receptor agonists [14,15]. Central alpha-1-adrenoceptors regulate the degree of excitability of target neurons since alpha-1-mediated responses to noradrenergic pathway stimulation or microiontophoretic application of alpha-1-receptor agonists last several hundreds of milliseconds [14,24]. Such membrane effects probably account for the facilitatory action of norepinephrine since depolarization would tend to bring the neuron closer to the threshold potential required for action potential generation.

Neurons in the dorsal raphe nucleus are preferentially activated by alpha-1-adrenoceptor agonists [4] and inhibited by alpha-1-receptor antagonists [14]. Baraban *et al.* [4] have suggested that the norepinephrine terminals present in the dorsal raphe of the rat, mediate a tonically active adrenergic influence upon which the firing of 5-HT cells depends. Heym *et al.* [9] have reported that the adrenergic influence on serotonergic raphe neurons in the freely moving cat is very small. This is in contrast to the reported complete suppression of unit activity produced by alpha-adrenoceptor blockade in anesthetized rats [4,14]. The disparity between the results of studies in cats and rats could be a species difference. Heym *et al.* [9] had earlier suggested that this disparity might arise since their studies were from behaving cats whereas those of Baraban and Aghajanian [4] were from

TABLE 2

COMPARISON OF *IN SITU* VERSUS *IN VITRO* ELECTROPHYSIOLOGICAL RECORDING TECHNIQUES

Advantages	Pitfalls
In situ	
1. Intact animals—hence most homeo-static mechanisms still present	1. Anesthetic agents may alter physiology
2. Synaptic activation possible	2. Concentration of drug reaching receptors unknown
3. Antidromic invasion and hence neuron identification possible	3. Mechanically unstable—hence intracellular studies difficult
4. Natural environment for neurons—no artificial cerebrospinal fluid	
In vitro (brain slices/cell culture)	
1. Mechanically stable—stable recording	1. Trauma—of slicing
2. Anesthetic agents not present	2. Afferent and efferent pathways may not be intact
3. Drug concentration at receptor site "known"	3. Homeostatic mechanisms absent
4. Visual control of electrode placement—receptor "hot spots" can be mapped	4. Artificial cerebrospinal fluid may lack transient alterations in modulators, e.g., hormones
5. Correlative electrophysiological and biochemical/anatomical techniques can be employed on same tissue	

anesthetized rats. However, VanderMaelen and Aghajanian [29] have recently shown noradrenergic activation of presumed serotonergic dorsal raphe neurons in rat brain slices in the absence of any anesthetic agents.

Inhibitory actions of norepinephrine have been reported at several central beta-adrenoceptors, e.g., hippocampus [25] and the cerebellum [10]. Norepinephrine-containing fibers from the pontine nucleus locus coeruleus synapse on the cerebellar Purkinje cell [6]. Application of norepinephrine by microiontophoresis or stimulation of the locus coeruleus inhibits the spontaneous discharge of the cerebellar Purkinje cell [12]. β-Adrenoceptor antagonists such as sotalol antagonize the inhibitory actions of norepinephrine. The inhibitory effects of norepinephrine on rat cerebellar Purkinje cells are mediated by cyclic AMP [11]. The transmembrane responses to norepinephrine and to cyclic nucleotides both involve a novel type of hyperpolarization—the hyperpolarization that occurs is associated either with no change or with significant increases in the membrane resistance [26]. It has also been reported that norepinephrine administered iontophoretically (at doses which elicited no change in spontaneous activity) can augment inhibitory responses of Purkinje cells elicited either by electrical stimulation of convential afferent pathways or by iontophoresis of gamma-aminobutyric acid [8,21] suggesting that this catecholamine can improve signal-to-noise ratios. It has been reported that the noradrenergic facilitation of GABA-induced inhibition of Purkinje neurons is mediated by the activation of cerebellar beta-1-adrenoceptors located on Purkinje neurons [30].

For many years, it has generally been assumed that receptors were constant, static elements in a system. As a consequence, single unit recordings have been conducted for a few decades to examine the effects of various drugs on single neurons from acute animal preparations. That this approach constitutes an oversimplification, is suggested by observations such as desensitization, denervation supersensitivity, and developmental changes in neuronal physiology. These and other observations constitute the study of receptor plasticity (i.e., up and down regulation). This has given rise to the concept that receptors and receptor processes are finely and sensitively tuned to their ambient environment. As a consequence, chronic single unit recording in the CNS of awake, behaving animals need to be conducted. Electrophysiological studies of assessment of long-term changes in receptor sensitivity are now being vigorously pursued. Any alterations in response that might be observed with electrophysiological techniques, cannot always be definitively ascribed to changes in receptor properties. First, electrophysiological techniques cannot discriminate between alterations in *de novo* synthesis of receptors (i.e., absolute numbers of receptors) and alterations in receptor affinity. Subsensitivity and supersensitivity of a receptor system, are at best functional definitions, and sensitivity changes do *not* necessarily reflect corresponding alterations in the absolute number of receptors. Dissociations between receptor number and sensitivity of receptor systems are probably reflecting different stages in the process of plasticity or degree of coupling to effectors. The regulation of the receptor number and the efficacy of their coupling to their effectors represent functionally important steps in the transmembrane regulation of neuronal signaling. Second, since electrophysiological techniques measure a neuronal response which is removed many steps from the initial interaction at the drug receptor, it is conceivable that a given treatment does *not* affect receptor properties or absolute numbers but profoundly alters an intermediate event. In the latter case, measurement of the electrophysiological response would be inadequate to distinguish between changes in receptor properties per se and intervening intermediate steps that trigger the measured electrophysiological parameter.

TABLE 3

EQUIPMENT ESSENTIAL FOR RECORDING BIOELECTRICAL SIGNALS FROM SINGLE NEURONS

Equipment	Comments
Electrodes (glass/metal)	Main criterion is that tip size be smaller than neuron being recorded
Amplifier	Bioelectrical signals from *single* neurons have to be amplified to be recorded
Oscilloscope	Permits visualization of electrical characteristics of neurons, e.g., waveform, frequency, duraion, amplitude
Window Discriminator	Filters signals and permits discrimination of signal from noise
Chart Recorder	Permits graphic display of signal
Microdrive	Permits placement of electrodes
Additional equipment *not* listed:	stimulator; on-line computer/FM recorder; audiomonitor; iontophoresis machine.

In intact animals, receptor properties can be studied using parenteral or local (iontophoresis/pressure ejection) application techniques. Local application techniques, e.g., microiontophoresis, avoid many problems of parenteral administration by circumventing somatic effects, liver metabolism, and the blood-brain barrier, but even iontophoretic applications can affect elements of the nervous systems besides the neuron being studied. Artifacts such as local anesthesia, pH, current effects, have to be guarded against. Typical electrophysiological equipment required for studying neuroreceptor properties is outlined in Table 3. Electrophysiological quantification of neuroreceptor properties can be conducted using extra- or intracellular recording techniques. In either case, the response measured (e.g., frequency of discharge; ionic currents; membrane potential) is frequently removed several steps from initial receptor activation and thus it represents the end result of receptor activation. Electrophysiological quantification of receptor sensitivity can be accomplished by constructing dose-response curves. In studies employing parenteral drug administration, increasing doses of receptor subtype selective drugs are injected at preset intervals so that a cumulative dose-response curve is obtained. If the discharge rate of a central neuron is the parameter being studied, then the percent change in firing after each dose, is determined by comparing the response after each injection to that for an identical period before injection. In this case, cumulative log-dose response curves for the actions of agents on neuronal activity can be constructed and the ED_{50} calculated [14]. For quantification of neuronal sensitivity to locally applied agents, e.g., iontophoresed agents, IT_{50} values for each agent are calculated. An IT_{50} value represents the product of the current (I) applied through a drug barrel and the time (in

seconds) required to alter neuronal firing to 50% of the control (baseline) rate (T_{50}). T_{50} values at a given current are estimated from plots of the percent change of firing versus time after the ejection current is turned on. Using this IT_{50} paradigm, very sensitive neuronal responses are reflected by very low $I \times T_{50}$ numbers, while less sensitive responses are expressed by larger $I \times T_{50}$ values. A similar paradigm can be employed for agents applied by pressure ejection [17, 18, 19]. Regardless of the neuronal response being measured, electrophysiological measurements of ED_{50} and IT_{50} values from single identified central neurons, provide an extremely useful index of neuroreceptor plasticity. This data is all the more meaningful when obtained from models of pathophysiological conditions such as hypertension, "aging," seizure disorders and chronic drug treatment.

The ideal that is still being sought is assaying the sensitivity of a single population of receptors on single identified neurons in behaving (unrestrained) animals. Electrophysiological data obtained from such animals with intracellular (!) recording techniques before, during, and after chronic drug exposure would yield very significant information. Finally, it should be remembered that the concept of a receptor subtype is not new. What is new is the increasing amount of fanciful interpretation that results of different techniques have suggested. The rush to interpret anamalous results in terms of new receptor subtypes is where scientific discretion will have to be exercised.

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