NEURON-BASED SENSORS FOR BIOCHEMICAL QUANTITATION

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ABSTRACT

Using intracellular recording from freshly dissected *Limnea stagnalis* neurons and cultured clones of mouse neuroblastoma, N-18 and N1E-115, linear relationships between several analyte concentrations and neuron electrical properties, such as action potential may be obtained. This demonstrates the suitability of neurons as primary transducers in neuron based sensors.

INTRODUCTION

The major problems in reliably determining *in vitro* or *in vivo* concentrations of antibodies or antigens, and for that matter any hormone, protein, ion, toxin, drug, or hazardous substance, are the lack of fast, reusable and accurate sensing devices. To date, many solutions have been tried, yet most are still unsatisfactory [1]. In this project, a new approach to sensing is being investigated in which the long term goals are to develop biochips which will be used to monitor electrical activity of neurons and later excitable synthetic membranes on exposure to analytes. The proposed sensing devices will allow one to take advantage of the specificity, sensitivity, and speed of response characteristic of neurons.

The ability of neurons to sense environmental changes is exceedingly specific and exquisitely sensitive [2]. To these characteristics one can add strikingly rapid initial response times. For the central nervous and endocrine systems and occasionally for the immune systems, these are often as short as milliseconds [3]. In addition chemical concentration information is encoded in the digital AP response of repetitively firing neurons. The availability of reliable digital signals has been cited by Brignell [4] and Koelman and Regtien [5] as the key factor required for future development of microsensors, because such responses are microprocessor compatible, and can easily be applied in bus-organized data acquisition systems.

However, for any neuron, there is paucity of *in vitro* data regarding the most suitable electrical property that correlates analyte concentration, analyte sensitivity limits, response time and response reproducibility. Without this information it remains unclear as to the suitability of neurons as a primary transducers. The purpose of this paper is therefore to report preliminary results obtained in our laboratory to verify the above concept for ethanol and serotonin, a neurotransmitter. Also initial developmental work that will lead to testing cultured neurons for the detection of monoclonal antibodies is reported.

MATERIALS AND METHODS

Three neuron types were used. The first were freshly dissected out of a pond snail *Limnea stagnalis* and the second and third were clones of the C1300 A/J mouse neuroblastoma, N-18 and N1E-115. The preparation of snail neurons involved removal of the visceral and right parietal ganglia from the circumoesophageal nerve ring using the methods of Byerly and Hagiwara [6].

The ganglia were transferred to a continuous flow recording chamber and exposed to varying concentrations of ethanol and serotonin in *Limnea* saline solutions. Cells were impaled with glass microelectrodes and stimulated to produce action potentials (APs) by passage of current through a bridge circuit from a Dagan 8100 high-input impedance amplifier (Dagan Corp., Minn., MN). Signals were monitored using a storage oscilloscope (Tektronix Corp., Beaverton, OR) and stored for later analysis on a DC tape recorder (Vetter Co., Rebersburg, PA). Cells selected for analysis were limited to those which regularly induced spike discharges of amplitudes greater than 50 mV. Repetitive firing rate was based on the interspike interval for the first four APs, obtained from cells induced by passage of a 1.0 S current pulse with a 0.25 Hz repetition rate. Responses of different neurons were compared by normalizing firing frequency values to the baseline (no analyte) response at a given current level and plotting the results as a function of concentration.

Cultured neurons were obtained from Dr. M. Nirenberg of NIH. These clones which are electrically active when differentiated with common antimitotic agents [7 & 8], were cultured after the procedures of Miyake [9] and Moolenaar and Spector [7]. Dimethylsulfoxide (DMSO) treatment and serum reduction to 2% levels were applied to induce differentiation in N-18 and N1E-115 respectively. In some cases Aminopterin (ATP), an agent that arrests DNA synthesis was administered. For intracellular recoding, the procedure described above for snail neurons was followed with the exception that the recording media was made up of 50% Hanks salt solution and 50% tissue culture media, without serum. Polyspecific serum or polyclonal antibodies (PoAbs) were produced by hyperimmunization of New Zealand white rabbits with differentiated neurons. PoAb titers were quantified by suspension enzyme-linked immuno-sorbent assay (ELISA) technique of Aydintug [10].

RESULTS AND DISCUSSION

Seven cells were exposed to ethanol in the 0.2 - 1.0 M range. Some cells showed excitatory effects with increasing concentration as shown in Figure 1. The higher the ethanol concentration, the higher the firing frequency. In Figure 2, plots of normalized firing frequency vs. ethanol concentration with 95% confidence interval bands on the mean values, shows three distinct categories: Group 1 with a strong excitatory response, Group 2 with a weaker response and Group 3 with no response. Preliminary data for a single neuron from the visceral ganglia at 2.9 nA was obtained for serotonin application. The neuron was exposed to two series of successively higher levels of serotonin concentrations of 0.1, 0.5 and 1.0 mM, followed by a saline rinse. One can conclude from serotonin as well as ethanol data that these compounds will elicit reversible concentration dependent responses for certain neurons. Linear correlation between analyte concentration and a property of a neuron demonstrates in a preliminary way the feasibility of the sensor concept. The above results were obtained with randomly selected neurons.

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Current work is focused on identification of neurons types that consistently demonstrate the desired response profiles as well as demonstrating an expanded scope of applications, such as monoclonal antibody detection.

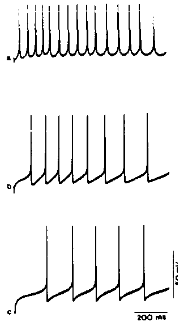

Figure 1. Effects of ethanol on the firing frequency in *Limnea* neurons (stimulating current was 0.8 nA).

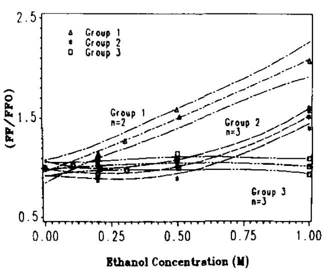

Figure 2. Normalized firing (FF/FFO) at 0.7 nA. Outer lines for each group of cells represents 95% confidence limits on the mean values.

Neuroblastomas are seen as highly suitable preparations for this work for several reasons. First, they are cloned lines of mammalian excitable cells [11 & 12] that exhibit the voltage-sensitive ionic conductances responsive to various neurotransmitters [13] and chemical stimulants (e.g. low molecular weight alcohols, ketones, aldehydes and carboxylic acids as shown by Kashiwayanagi and Kurihara [13 & 14]. Hence, they will serve as an excellent model for sensing specific aqueous stimulants at a speed and sensitivity characteristic of excitable cell membranes. Secondly, methods exist for modifying the membrane structure of the neuron clones [15] to vary the response profile toward the same chemical species. This will provide greater specificity toward certain stimulants and the potential to characterize complex mixtures. Finally, since neuroblastomas are amenable to culturing, they will provide uniform populations with minimal cell to cell variation. Generation of repetitive APs in neuroblastomas has been shown to be to a large extent a function of differentiation [9 & 16] which can be induced by reducing serum concentration in the growth medium [17], the use of phosphodiesterase and DNA synthesis inhibitors [18], and by glucorticoid (steroid) treatment [19].

We have been successful in culturing cells that are capable of generating APs. Active cells were obtained with administration of APT, a compound used to kill dividing cells by arresting DNA synthesis. Hence, APT aids identification of neurons that have lower resting potentials on impalement with glass microelectrodes.

The lower potentials are characteristic of fully developed membranes [20] and are necessary for AP generation [9]. For all the cells that were impaled, the resting membrane potential was observed to increase to zero at a rate which was faster in some cells than in others. Miyake [9] reported similar observations due to weak cell membranes that are injured during glass microelectrode impalement. However, he showed that stronger cell membranes resulted by increasing Ca^{++} and Mg^{++} concentrations in the tissue culture media, providing repetitively firing neurons with long term stability.

ELISA assays were performed to compare the amount of antibody bound to N1E-115 cells for rabbit serum before and after three successive immunizations. A 52% increase in the titers of PoAb against the N1E-115 cells was observed. Soon cultured neurons will be subjected to PoAbs and it is expected that concentration dependent responses will be observed. Also studies by this research group are in progress to repeat experiments by including other antimitotic agents and using APT and higher Ca^{++} and Mg^{++} levels, to optimize tissue culture procedures. Besides the above, work is also in progress to fabricate micromachined polyimide structures for containing the neurons. It is expected that extension of this work will involve building biochips that will contain the neuron as well as integrated circuits.

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