CANSTAT-4: A four channels potentiostat for the on-line monitoring of catecholamine secretion.

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Amperometric techniques have been used for the analysis of the secretory responses from perfused adrenomedullary tissues like whole adrenal (cat [1], rat[2]) or cultured bovine cells. However, all of the commercially available equipments are electrochemical detectors used for HPLC (BAS, Metrohm). However, these apparatus are single-channel and the use of simultaneous recording will require the use of additional detectors with an increase in the price and bench space. Another disadvantage is the lack of proper software for the stimulation of the tissues and for multi-channel acquisition of secretory signal(s).

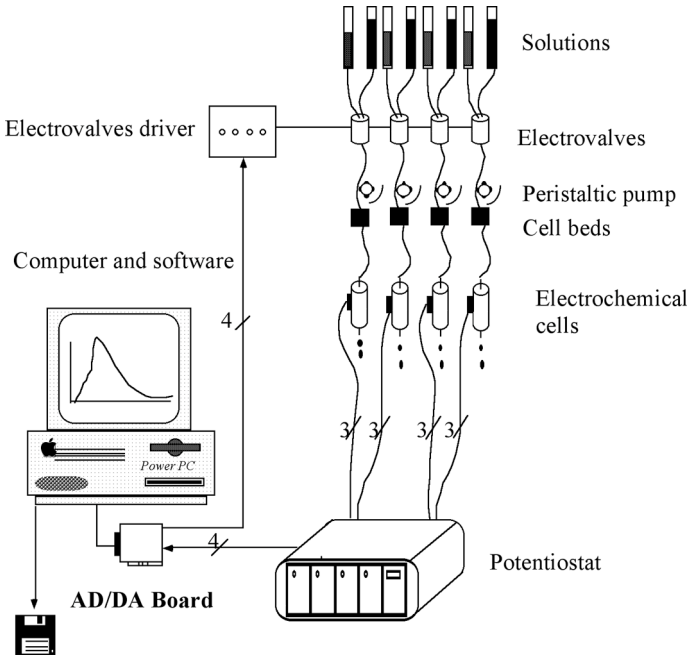

Figure 1. CANSTAT-4 setting-up. Basal incubating solutions or chemical stimuli are selected by the electro-valves. These valves can be manually operated or by a TTL signal generated by a computer digital output. A four-channel peristaltic pump is continuously perfusing the cell chambers or adrenal glands. The emanating fluid is passed through the electrochemical cells for catecholamine detection. The potentiostats send the analogic signal (± 12 V) to a computer through a 12-bit AD/DA board (PCI-1200, National Instruments)

We introduce here the CANSTAT-4, which has been designed and constructed in our lab to satisfy all of these

requirements. This is an integrated system for the simultaneous recording of the secretion of catecholamines from four perfused adrenal glands or cell beds.

CANSTAT-4 system includes: i) four potentiostat amplifiers, caged in individual shielded plug-in units, ii) new designed compact three-electrodes electrochemical cells, iii) computer-controlled drug administration by an electro-valve driver, iv) software for acquisition, v) variable applied voltage driven by the computer for voltammetry and vi) control of chemical stimulation protocol. Our system uses a graphic computer language (LabVIEW™, National Instruments, Austin, TX, USA) that can operate either under Macintosh or Windows environments.

The system requires some additional equipment: a peristaltic pump, a computer and a AD/DA board. Optionally, the program can trigger an electrical stimulator for the study of splanchnic nerve-chromaffin cell synapse.

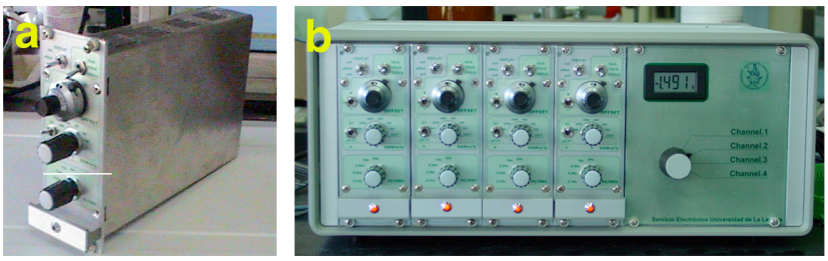

Figure 2. General view of the potentiostat amplifier. Panel **a** shows an individual plug-in module On panel **b** is shown the front view of the equipment with the four modules inserted and the common display. External dimensions 42 x 25 x 18 cm.

One important feature of the system is its modular structure. Hence it can operate with 1 to 4 channels independently and individual modules can be removed from the main box without alter the functioning of the other channels. Each module is a plug-in shielded box to avoid electromagnetic interferences (figure 2a). The oxidation/reduction voltage can be set from either an internal variable resistor or externally applied by the computer AD/DA

board. The front panel contains the gain, offset and filter controls whereas in the back of the main box are placed the plugs for the electrochemical cells and for the input/output terminals. A common display allows monitoring the electrode potential, the offset applied to the output signal and the output value for every channel (Figure 2b).

The electro chemical cells have been designed compact, light and small in order to be accommodated near the tissues thus reducing the liquid dead space. Each electrode can be removed for cleaning or replacement (figure 3a). The valve controller is a compact cage intended to be placed near the peristaltic pump. It can be used either in a manual or in a computer-operated mode.

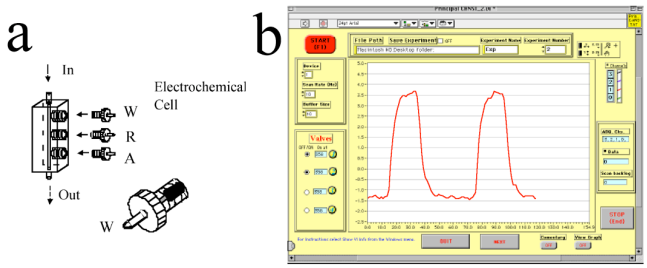

Figure 3. Some novelties of the CANSTAT-4. a) the system includes compact electrochemical cells for three electrodes: working (W, glassy carbon); reference (R, silver chloride) and auxiliary (A, stainless steel). Each electrode is built in a plastic screw that can be removed independently. **b)** the computer program allows the continuous acquisition and display of data. In the picture, a single channel record of catecholamine secretion shows two secretory peaks obtained after the stimulation of a cell bed (2 millions) with 30 µM DMPP for 15 s.

Although data obtained from CANSTAT-4 can be displayed by any acquisition program or chart recorder, we have created a specific system that combine data acquisition with stimulation protocols. The software designed for the CANSTAT-4 has been written using LabVIEW™. Data from all channels (oxidation/reduction currents) are online displayed on the computer screen and quantified in nA. The program returns and saves binary files ready for their analysis using Igor Pro (Wavemetrics, Lake Oswego, OR, USA).

The CANSTAT-4 constitutes a new system for the multiple online analysis of catecholamine secretion from adrenomedullary tissues. It opens the use of these tissues for simultaneous measurements useful for a wide range of purposes including drug testing.

ACKNOWLEDGMENTS

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