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THE USE OF ROBOTS AND COMPUTERS IN THE ORGANISATION OF STUDIES ON THE CIRCADIAN VARIATION OF β_2-ADRENOCEPTOR SITES IN PERIPHERAL MONONUCLEAR LEUCOCYTES

B. Liebl, E. Haen, U. Romacker, P.-T. Nguyen and J. Remien

Walther-Straub-Institute of Pharmacology and Toxicology, Nussbaumstr. 26, D-8000 Munich 2, F.R.G.

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Abstract—We have developed a partially automated method for the performance of equilibrium radioligand binding studies which is applied by our group in investigations on circadian variations and stimulation studies on β_2-adrenoceptor sites in human peripheral mononuclear leucocytes (pMNL). Using a Tecan Robotic Sample Processor, binding assays with 12 concentrations of ^{125}iodocyanopindolol (1–150 pmol/l, total binding in triplicates, unspecific binding in the presence of 10^{-5} mol/l timolol in duplicates) are prepared automatically with all titer tubes per experiment arranged in a microtiterplate-sized rack. After incubation in a waterbath for 2hr at 37° C, the whole rack is centrifuged at 5000*g* and transferred back to the lab robot. Bound radioactivity is separated from the unbound ligand by removing the supernatant by the machine. The radioactive counts are evaluated using personal computers. The lab robot enhances reproducibility of experimental results and frees lab workers from time-consuming pipetting jobs. Radioactive exposure is minimized to the time preparing the radioligand working solution and transferring the sample tubes from the robot to the waterbath, to the centrifuge and back to the robot. The variability of our software allows easy adaptation to other binding studies with intact cells.

Key words—Sampling robots, circadian studies, equilibrium binding studies.

Introduction

Equilibrium radioligand binding studies are widely used for investigations on the expression of neurotransmitter and hormone receptors. To study circadian variations and other effects along a timescale (drug–response studies), multiple experiments are necessary. We are interested in variations in the expression of β_2-adrenoceptor sites in human peripheral mononuclear leucocytes (pMNL) (1), implying experiments at 4 hr intervals and 30, 60, 120, 180, 240 and 360 min after a stimulus, e.g. allergene provocation or steroid application. Altogether, up to 14 experiments have to be performed within 36 hr for each subject.

Since different experimenters are engaged in every set of experiments, validity is highly dependent on the reproducibility of experimental results. The pipetting tasks especially are prone to systematic and random errors, due to the varying manual skill of different investigators.

Using a pipetting robot and IBM compatible PCs, we therefore developed a partially automated method which is less laborious and minimizes radioactive exposure of lab workers.

Materials and Methods

A receptor binding assay (in triplicate) is used with 12 concns of ^{125}iodocyanopindolol (^{125}ICYP) in the concn range of 1–150 pmol/l. Unspecific binding (in duplicate) is determined in parallel samples with 10^{-5} mol/l of unlabled (-)-timolol (displacer). Altogether 60 specimens per experiment have to be prepared, with varying volumes of radioligand and buffer and a constant volume of timolol (20μl) and cell-suspension (100μl) to achieve a total reaction volume of 1000μl.

We are using a Tecan Robotic Sample Processor Model 5032 (dual arm system) for preparation, employing a software especially designed by our group for this type of experiment (for description, see later). Pipetting starts at the time of the first blood specimen being drawn and is executed parallel to preparation of MNL by density gradient centrifugation (2, 3). Automatic

pipetting of one experiment takes about 10 min. All titer tubes (1 ml, Biorad) are arranged in a microtiterplate-sized rack. Experiments are started by adding 100 μl pMNL suspension in a concentration of 5×10^6 cells/ml to each of the 60 specimens. After incubation in a waterbath for 2 hr at 37°C the experiment rack is centrifuged at 5000*g* for 10 min in a Eppendorf–Hermle® centrifuge, and transferred back to the lab robot. Bound radioactivity is separated from the unbound ligand by removal of the supernatant by the machine (Table 1).

The radioactive counts are evaluated using personal computers. To determine the number of high affinity binding sites (B_{max}) and their equilibrium dissociation constant (K_d), data are mathematically fitted to a binding equation for one class of saturable high affinity binding sites and a second class of low affinity binding sites (2, 3) using a computer-based nonlinear iteration procedure and a commercially available program named Enzfitter® (Elsevier, Amsterdam).

The described method has been applied by our group in several clinical studies for results, see reports presented elsewhere.

Assay pipetting software

Our program handles pipetting parameters of 1–6 saturation experiments at a time. Up to 24 radioligand concns in a range over 4 orders of magnitude may be defined per experiment. An additive (e.g. antioxidants) may be supplemented to all samples uniformly. Pipetting may be performed alternatively in 1 of 3 different tube or rack types, respectively, with total sample volumes between 200 and 2000 μl (additive: 10–100 μl; displacer: 10–50 μl; tissue: 50–200 μl). The pipetting sequence can be altered, automatic pipettes may be switched on or off so that certain pipetting steps may be performed by hand or at a later point of time (e.g. the tissue pipette, if samples are prepared for an experiment which is to be started later by tissue addition). All parameter inputs are menu controlled (Figure 1). In dependence on substance-specific constants (molecular weights, specific activity) and experiment-specific variables (volumes, concns), required reagent dilutions (radioligand, displacer, additive) and titertube arrangements are displayed. The pipetting steps

Table 1. Comparison of time consumption and exposure to radioactivity with and without use of lab robot and personal computers for a single experiment

Task	Without lab robot and computers: total time (min)	working time (min)	radioact. exposure	With lab robot and computers: total time (min)	working time (min)	radioact. exposure
Cell preparation by density gradient centrifugation	120	30	–	120	30	–
Inscription of tubes	15	15	–	–	–	–
Arrangement of tubes in racks	5	5	–	5	5	–
Calculation of dilutions	10	10	–	–	–	–
Preparation of stock dilutions	10	10	+	10	10	++
Pipetting of 60 tubes (radioligand, buffer, displacer, cells)	40	40	++++	10	2	–
Transfer to waterbath+ incubation	120	5	+	120	5	+
Transfer to, loading of centrifuge Centrifugation, unloading of centrifuge	12	2	+	10	1	+
Separation of bound from free radioactivity	20	20	+++	10	3	–
Loading of radioactivity-counter and counting	125	5	–	125	5	–
Raw data analysis	60	60	–	5	5	–
Nonlinear iterative curve fitting	not possible		–	10	10	–
Sum	537	202		425	76	

Exposure: – = none, + = low, ++++ = high.

Method-File-Nr.1: β-AR, 12 concns/B.Liebl

Saturation: Settings			
Title	β-adrenoceptors	Date 05.03.1990	
Radioligand	ICYP $t_{1/2}$ 60 d	molecular weight	411
charge-Nr.	lot 195 age 55 d	reference date	15.01.1990
concn	1.8E+06 Bq / 500µl	specific activity	6.7E+16 Bq/mol
Tissue	intact pMNL	pipetting	by hand
Displacer	(-)-timolol	concentration	1.0E-04 mol/l
molecular weight	423	pipetting	by machine
Additive	———	concentration	———
molecular weight	———	pipetting	———
Rack, tubes MTP2 / 1 ml		*Arrangement* 1 experiment/rack	
total sample volume 1000µl		displacer-vol./sample 20µl	
tissue-volume/sample 100 µl		additive-volume/sample ———	

<⊥ =continue SHIFT<⊥ =back F1=menu F3=input F4=print ESC=done,abort

Method-File-Nr.1: β-AR, 12 concns/B.Liebl

Saturation: concns, replications					
Replications/experiment		triplicates	< duplicates, triplicates >		
Concentrations/experiment		12	< 1-24 >		
Number of experiments		3	< 1-6 >		
Concns					
1.	1.0E-12	mol/l	7.	1.3E-11	mol/l
2.	1.5E-12	mol/l	8.	2.0E-11	mol/l
3.	2.0E-12	mol/l	9.	3.0E-11	mol/l
4.	3.0E-12	mol/l	10.	4.5E-11	mol/l
5.	4.5E-12	mol/l	11.	7.5E-11	mol/l
6.	7.5E-12	mol/l	12.	1.5E-10	mol/l

<⊥ =continue SH<⊥ =back F1=menu F2=save F3=input F4=print F9=get, F10=save exp. ESC=done,abort

Figure 1. Hardcopy of two main input screens defining experimental parameters for pipetting by the sample robot.

in performance are displayed and may be printed on a hardcopy device together with exact time course informations. All parameters can be saved in so-called method files, loaded bypassing the input-functions, and may be copied, deleted or revised and saved anew. Another option allows a parameter-listing or the generation of an ASCII-format file which may later be brought together with the radioactivity measurements for data analysis.

Compared to the conventional rapid filtration method over glass fiber filters to separate bound from unbound radioactivity, the suggested centrifugation method saves material (no filters necessary), time and radioactive exposure of the experimentors. Again, employing the lab robot for this job leads to high reproducibility.

Since the input part of the lab robot program provides variability of all pipetting parameters, the reported method may easily be adapted to binding studies on other binding sites in intact cells.

Discussion

Without the lab robot one person was busy for 1 day preparing a circadian study with 7 experiments (inscription of titer tubes, arranging them in racks, making reagent dilutions, pipetting jobs). To study the effect of a stimulus (allergen provocation, steroid exposure), a second technician was needed. By employing the lab robot preparation time is reduced to loading the method file, filling the racks with titer-tubes and preparing reagent dilutions according to the

instructions of the program (Table 1). Automatic sample processing enables an optimal reproducibility of sample composition in every experiment.

Radioactive exposure of the workers employed is minimized to the time of transferring the sample tubes from the robot to the waterbath, to the centrifuge and back to the robot. After preparing the radioligand working solution, no further direct contact with radioactivity is necessary.

References

1. Haen E., Emslander H. P. Liebl B. Langenmayer T. and Remien J. *Atemwegs- und Lungen.- krankheiten* **15**, 387–388, 1989.
2. Anhäupl T., Liebl and Remien J. *J Recept Res* **8**, 47–57, 1988.
3. Liebl B., Anhäupl T., Haen E. and Remien J. *Naunyn Schmiedeberg's Arch Pharmacol.* **338**, 523–528, 1988.
4. Haen E., Langenmayer I., Pangerl A., Liebl B. and Remien J. *Klinische Wochenschrift* **66**, 579–582, 1988.