INIBSA/Instituto de Farmacología Teófilo Hernándo
Pharmacological profile of IQB-9302, a new local anaesthetic

REPORT 99/2

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EFFECTS OF IQB-9302 ON VOLTAGE-DEPENDENT Ca^{2+} CHANNELS AND NEURONAL NICOTINIC RECEPTOR ION CHANNELS

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Effect of IQB-9302 on voltage-dependent Ca^{2+} channels and neuronal nicotinic receptor ion channels.

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DATE OF THE STUDY: April 1999 - May 1999

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Number of pages of the report: 8

The present study was performed in compliance with the rules and regulations of Good Laboratory Practices published by OECD (1981) and according to the Real Decreto 822/1993 BOE (May 1993). There were no incidences that could affect reliability of data.

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INTRODUCTION

As mentioned in previous reports, local anaesthetics like IQB-9302 or bupivacaine bind to a specific receptor inside the pore of the Na^+ channel in the nerves, blocking the entry of Na^+ ions. Nevertheless, we think that in the context of the study of the pharmacological profile of this new compound, it would be of interest to study possible additional blocking effects of IQB-9302 on voltage-dependent calcium channels or nicotinic acetylcholine receptor-associated channels.

With this purpose, we studied the effects of IQB-9302 and bupivacaine on $^{45}Ca^{2+}$ uptake into bovine chromaffin cells induced by K^+ depolarisation or by stimulation of nicotinic receptors with the agonist DMPP (dimethylphenyl-piperazinium iodide).

MATERIALS AND METHODS

Isolation and culture of bovine chromaffin cells.

Bovine adrenomedullary chromaffin cells were isolated following standard methods (Livett, 1984) with some modifications (Moro et al., 1990). Cells were suspended in Dulbecco´s modified Eagle´s medium (DMEM) supplemented with 5% foetal calf serum, 10 μM cytosine arabinoside, 10 μM fluorodeoxyuridine, 50 IU ml^{-1} penicillin and 50 μg ml^{-1} streptomycin. Cells were plated at a density of $5x10^5$ cells/well in 24-multiwell Costar plates and were used 1-5 days after plating. Medium was replaced after 24h and then after 2-3 days.

MATERIALS

IQB-9302.HCl batch nº 9454.001 was obtained from LEBSA. Dulbecco's modified Eagle's medium (DMEM; GIBCO); Collagenase from *Clostridium histolyticum* (Boehringer-Manheim). Bovine serum albumin fraction V, soybean trypsin inhibitor, cytosine arabinoside, fluorodeoxyuridine, 1,1-dimethyl-4-phenyl-piperazinium (DMPP), ethylene glycol-bis(β-aminoethyl ether) N, N, N', N'-tetraacetc-acid (EGTA), bupivacaine.HCl (Sigma); Percoll (Pharmacia); foetal calf serum, penicillin and streptomycin (GIBCO); scintillation fluid Optiphase Hisafe II (EGG Instruments); $^{45}Ca^{2+}$ (specific activity 10-40 mCi mg^{-1} calcium, American Radiolabeled Chemicals Inc.). All other chemicals were reagent grade.

SOLUTIONS

Krebs-HEPES solution of the following composition (mM) was used: NaCl 140, KCl 5.9, $MgCl_2$ 1.2 , $CaCl_2$ 1, glucose 11, HEPES 10, at pH 7.2 . IQB-9302.HCl and bupivacaine.HCl were dissolved in distilled water at a concentration of 10^{-2}M and at the moment of each experiment aliquots of these solutions were diluted in Krebs-Hepes solution to the desired concentration.

METHODS

Measurements of $^{45}Ca^{2+}$ uptake

$^{45}Ca^{2+}$ uptake studies were carried out in cells after 2-3 days in culture. Before the experiment, cells were washed twice with 0.5 ml of the Krebs-HEPES solution at 37ºC.

$^{45}Ca^{2+}$ uptake into chromaffin cells was studied by incubating the cells at 37ºC with $^{45}CaCl_2$ at a final concentration of 5 μCi ml^{-1} in Krebs-HEPES (basal uptake). This incubation was carried out during various times in the absence (control) or in the presence of the drugs and at the end of the incubation period the test medium was rapidly aspirated and the uptake reaction was ended by adding 0.5 ml of a cold Ca^{2+}-free Krebs-HEPES containing 10 mM $LaCl_3$. Finally, cells were washed 5 times more with 0.5 ml of Ca^{2+}-free Krebs-HEPES containing 10 mM $LaCl_3$ and 2 mM EGTA, at 15 s intervals.

To measure radioactivity retained by chromaffin cells, the cells were scraped with a plastic pipette tip while adding 0.5 ml 10% trichloroacetic acid, 3.5 ml of scintillation fluid (Optiphase Hisafe II, EGG Instruments) was added and the samples counted in a Packard beta counter. Results are expressed as % of $^{45}Ca^{2+}$ taken up by control cells.

ANALYSIS OF DATA

Data of $^{45}Ca^{2+}$ uptake in the absence (control) or the presence of the drugs were transferred to an Excel worksheet and were transformed into percentage considering 100% $^{45}Ca^{2+}$ uptake by control cells (in the absence of drugs). The data of all the experiments were included in the same Excel worksheet and mean $\pm$ SEM was calculated. IC_{50} values were calculated from a non linear regression analysis using ISI software, with a PC computer.

RESULTS

Fig.1 shows the dose-response curves for the effect of IQB-9302 on $^{45}Ca^{2+}$ uptake induced by 70 mM K^+ (A) or 100 μM DMPP (B) into bovine chromaffin cells. When cells were stimulated with 70 mM K^+, only at concentrations of 100 μM or above a noticeable blockade of the $^{45}Ca^{2+}$ entry could be seen, with an IC_{50} of 310 μM. If the $^{45}Ca^{2+}$ uptake was induced by the nicotinic agonist DMPP, the blockade was complete at 30 μM, and the IC_{50} was 9.3 μM.

In Fig. 2 the same type of experiments of Fig. 1 were repeated for bupivacaine. When cells were stimulated with 70 mM K^+ the effect of bupivacaine on $^{45}Ca^{2+}$ uptake was somehow more potent than that seen for IQB-9302, with an IC_{50} of 110 μM. Also bupivacaine was more potent that IQB-9302 in blocking the $^{45}Ca^{2+}$ entry induced by DMPP (IC_{50} = 0.32 μM).

DISCUSSION AND CONCLUSIONS

According to the results presented above, we can see that both IQB-9302 and bupivacaine could have an effect as blockers of voltage-dependent Ca^{2+} channels at concentrations of 100 μM to 1 mM, concentrations that are used for local application of these anaesthetics but that will never be reached sistemically. For nicotinic stimulation, the blockade is obtained at concentrations 10 times lower than for K^+, but still side effects derived from nicotinic receptor blockade of sympathetic ganglia should not be found after local applications of these compounds. It is woth noting that IQB-9302 blocked nicotinic receptors with an IC_{50} 30-fold lower than bupivacaine. This is an important difference that might have safety implications for the compounds. In the case of systemic accidental injection the hypotension derived from ganglionic blockade should be much less with IQB-9302 than with bupivacaine.

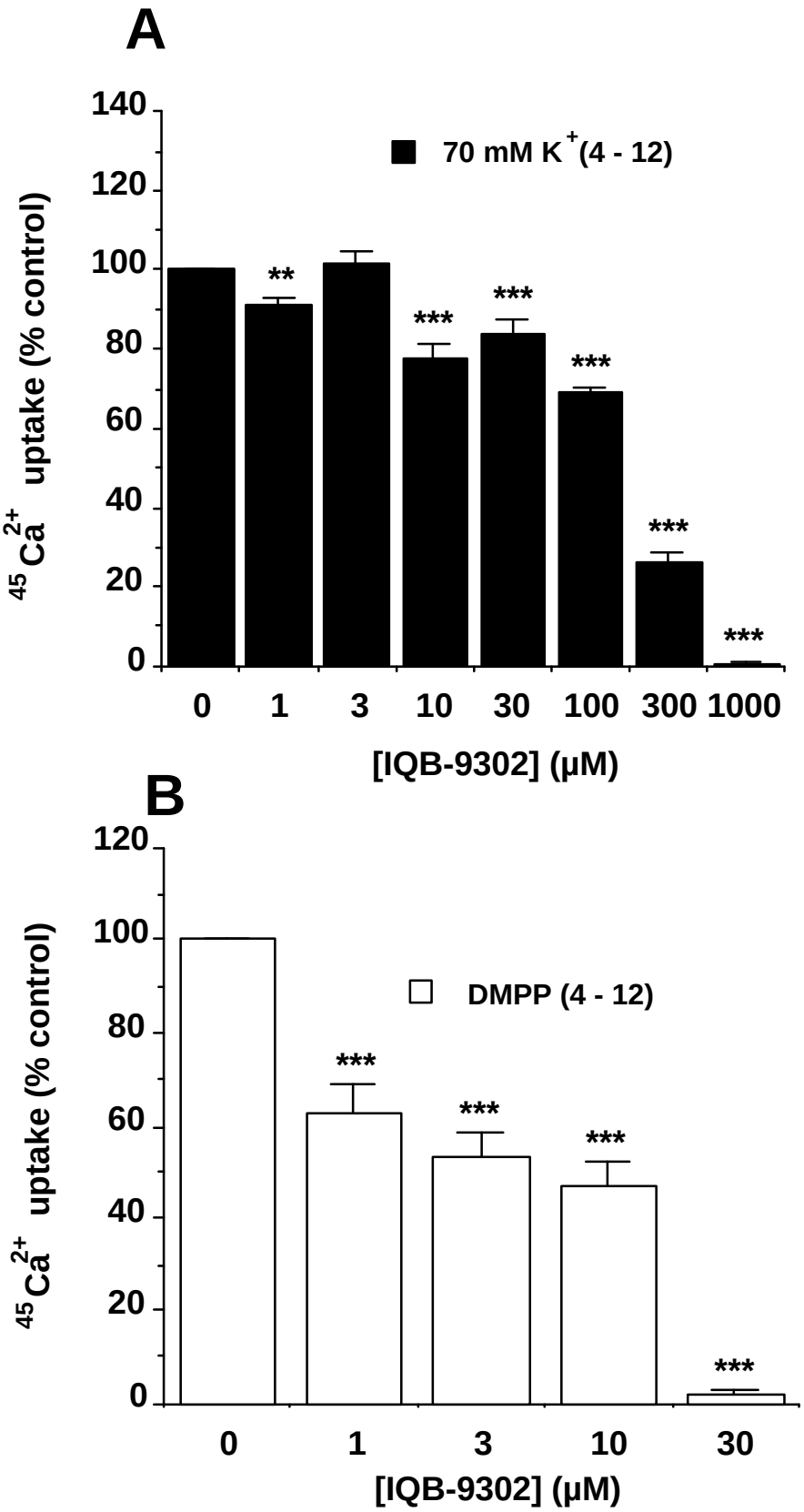

Fig.1.- Effects of IQB-9302 on $^{45}Ca^{2+}$ uptake into bovine chromaffin cells induced by 70 mM K^+ (A) or 100 μM DMPP (B). Data correspond to means$\pm$ SEM of the number of experiments shown in parenthesis.**P$\leq$0.01, ***P$\leq$0.001 with respect to their controls, in the absence of drug.

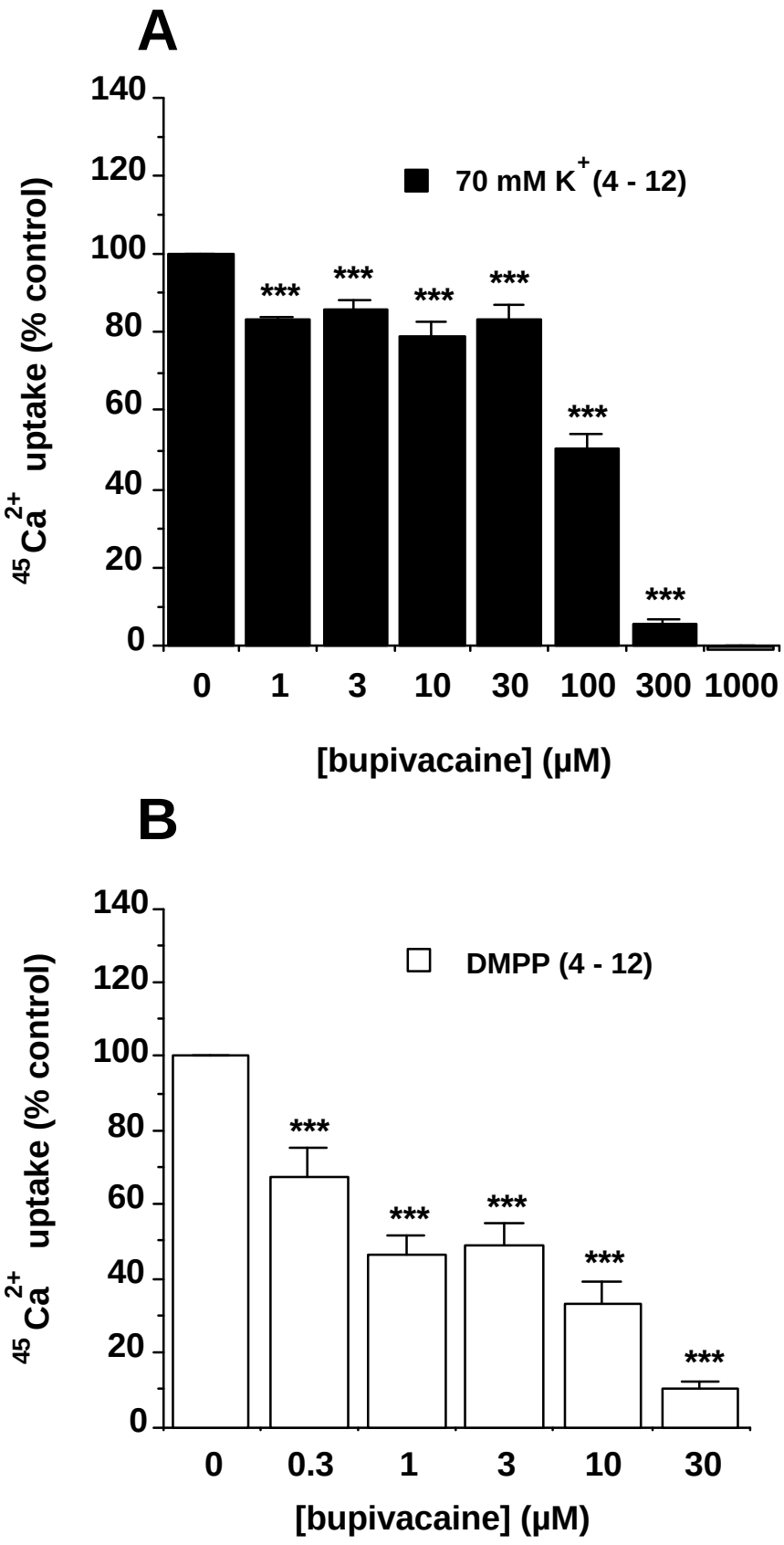

Fig.2.- Effects of bupivacaine on $^{45}Ca^{2+}$ uptake into bovine chromaffin cells induced by 70 mM K^+ (A) or 100 μM DMPP (B). Data correspond to means$\pm$ SEM of the number of experiments shown in parenthesis.***P$\leq$0.001 with respect to their controls, in the absence of drug.