DruQuaR

UNIVERSITEIT GENT

FACULTEIT FARMACEUTISCHE WETENSCHAPPEN

Bioactive peptides as potential new drugs: from analytics to tissue bath functionality.

M. Verbeken[1], S. Van Dorpe[1], R. Lefebvre[2] and B. De Spiegeleer[1*]

Ghent university: [1] DruQuaR, Harelbekestraat 72, B-9000 Ghent; [2] Departement of Pharmacology, De Pintelaan 185, B-9000 Ghent.
*Corresponding author: bart.despiegeleer@UGent.be (Ref.: 2010-260c)

INTRODUCTION

Peptides show great pharmaceutical potential as active drugs and diagnostics in several clinical areas such as endocrinology, obstetrics or oncology [1]. The search towards novel lead-peptides with a biological function has thus attracted renewed interest. Within a collaborative, multidisciplinary approach, several new peptides were identified with the potential of being new drugs or serving as a basis towards new drugs. Twenty-five peptides, selection based on a multi-criteria decision strategy, were tested in tissue baths using different smooth muscle preparations. A positive bioactivity was confirmed if a significant alteration of the smooth muscle contractility was induced by the addition of the investigated peptides. Analytical controls to establish the correct peptide structure responsible for the bioactivity have been performed as well, stressing the importance of purity control.

EXPERIMENTAL

Tissue-bath experiments

Isolated smooth muscle strips, maintained in temperature controlled Krebs buffer, were mounted between two platina electrodes attached to the mounting hook in glass tissue bath containers and connected to a force transducer to record smooth muscle contractions (Fig. 1). Four smooth muscle preparations were used for the functional screening of candidate bioactive peptides (Table 1).

Table 1: Isolated muscle preparations; experimental conditions.

Isolated preparations	Guinea pig ileum	Guinea pig trachea	Mouse vas deferens	Rat aortic ring
Pre-stretch (g)	0.7	2.0	0.2	2.0
Krebs buffer	Mg^{2+} containing	Mg^{2+} containing	Mg^{2+} free	Mg^{2+} containing
$T_{Krebs\ buffer}$ (°C)	36	36	31	36
Electrical stimulation	Yes	No	Yes	No

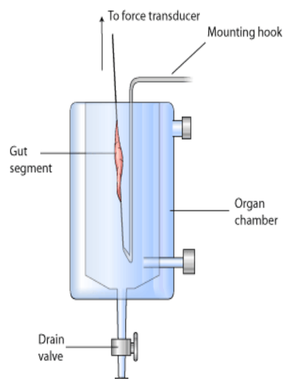


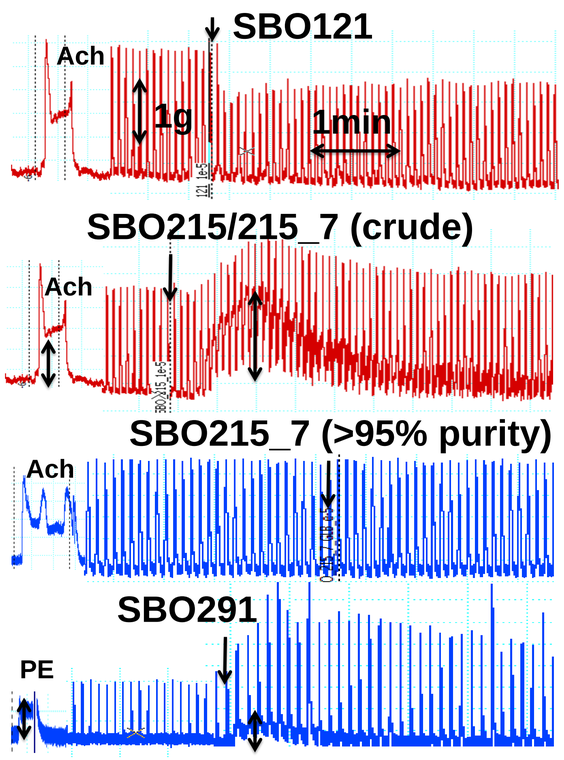
Fig.1: Tissue bath model with isolated smooth muscle strip.

Analytical control of bioactive peptides

The purity of peptides used for functionality testing ranged from crude to 98%. Stock and tissue bath samples of bioactive peptides were analyzed using HPLC UV-MS to identify the correct peptide structure responsible for the bioactivity. A C18 reversed phase column with gradient program (acetonitril/water mixture) and UV detection was used for quantification, while HPLC-ESI/iontrap MS^n was used for identification purposes [1].

RESULTS AND DISCUSSION

Twenty-five peptides were screened in four isolated smooth muscle preparations, three peptides were able to induce significant alterations of smooth muscle contractions: SBO121 and SBO215/215_7 (both in guinea pig ileum, gpI) and SBO291 (in mouse vas deferens, mVD) (Fig. 2).



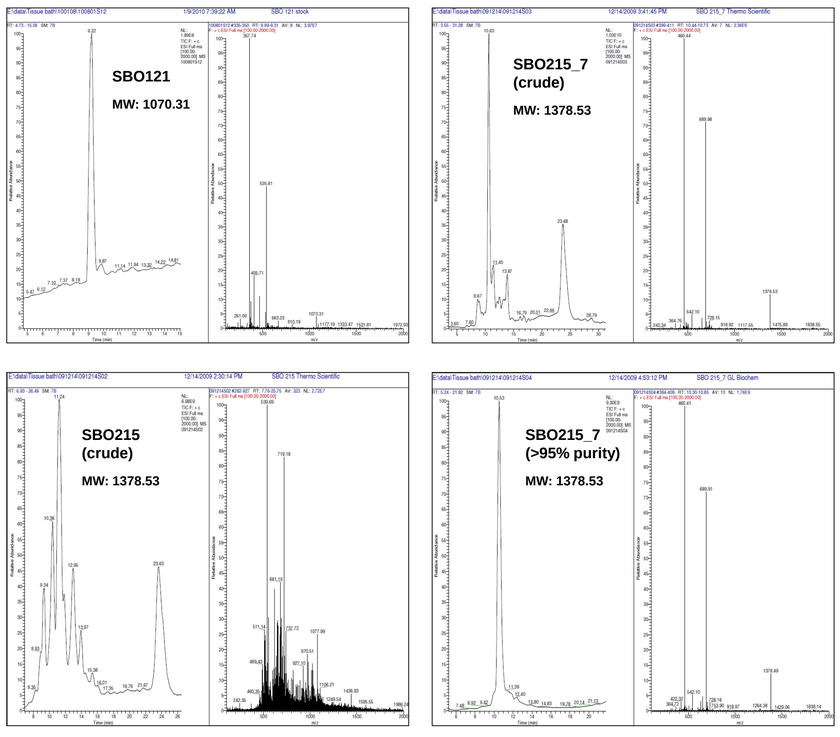


Fig. 2: Representative tracings showing the effect of SBO121 and SBO215/215_7 in gpI and SBO291 in mVD (left), and the apposite MS spectra (right). The repetitive contractions were induced by electrical field stimulation (EFS), the non-stimulated contractions by acetylcholine (Ach) and phenylephrine (PE).

Addition of SBO121 inhibited electrical induced smooth muscle contractions (Fig. 2-3, relative to EFS induced contractions before), while SBO215 induced baseline contractions without significant alterations of the magnitude of the EFS induced contractions in gpI. However, no baseline contractions were observed with pure SBO215_7 peptide. SBO291 exerts similar actions as SBO215, but on mVD.

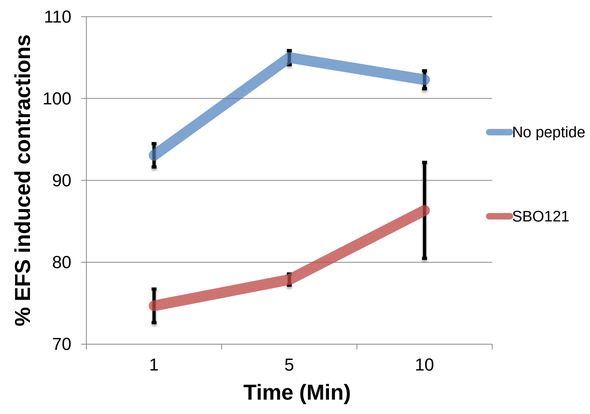


Fig. 3: SBO121 inhibition of EFS induced contractions.

CONCLUSIONS

Twenty-five candidate bioactive peptides were selected, based on a multi-criteria decision strategy, and screened in tissue organ bath experiments using four isolated preparations. Three peptides induced significant alterations of smooth muscle contractions: SBO121 and SBO215/215_7 (gpI) and SBO 291(mVD). However, it was observed that SBO215_7 (>95% purity) did not influence smooth muscle contractions, contrary to “crude” SBO215_7 peptide quality. Therefore, analytical controls are crucial to establish the correct peptide structure responsible for the bioactivity and to control the peptide purity.

REFERENCES

[1] Vergote, V., et al., Quality specifications for peptide drugs: a regulatory-pharmaceutical approach. Journal of Peptide Science, 2009. 15(11): p. 697-710.