# Synthetic mammalian C-type natriuretic peptide forms large cation channels

Joseph I. Kourie*

*Membrane Transport Group, Department of Chemistry, The Faculties, The Australian National University, Canberra, A.C.T. 0200, Australia*

Received 23 November 1998; received in revised form 11 January 1999

**Abstract We report the first evidence that synthetic human C-type natriuretic peptide-22 and the OaC-type natriuretic peptide-39(18–39), a 22 amino acid fragment of the OaC-type natriuretic peptide-39 from platypus venom, can function directly by forming a novel voltage-gated weakly cation-selective channel in negatively charged artificial lipid bilayer membranes. The channel activity is characterized by a tendency for inactivation at negative voltages, e.g. −60 and −70 mV, whereas at positive voltages the channel is fully open. The channel has a maximal cord conductance of 546 ± 23 pS ($n = 16$) and shows weak outward rectification. The sequence and the permeability ratios were $P^+_K : P^+_{Cs} : P^+_{Na} : P^+_{choline}$ 1:0.88:0.76:0.13, respectively. The addition of 50 mM $TEA^+_{cis}$ (a blocker of outwardly rectifying $K^+$ channels), 20 mM $Cs^+_{cis}$ (a blocker of inwardly rectifying $K^+$ channels) or 0.5 mM $glibenclamide_{cis}$ (a blocker of ATP-sensitive $K^+$ channels) to the *cis* chamber did not affect the conductance or the kinetics of the OaC-type natriuretic peptide-39(18–39)-formed channels ($n = 2$–5). It is concluded that the weak cation selectivity, large conductance and high open probability as well as their voltage dependency are consistent with the ability of these peptides to cause that loss of compartmentation of the membrane, which is a characteristic feature of adverse conditions that cause C-type natriuretic peptide-related pathologies.**



*Key words:* Natriuretic peptide; Large cation channel; Channel forming peptide; Signal transduction

## 1. Introduction

C-type natriuretic peptide (CNP), atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) form an important family of peptides which exhibit potent natriuretic, diuretic, hypotensive and vaso-relaxant properties [1–8]. C-type natriuretic peptides are distributed widely in the mammalian central nervous system, the brain, endothelial cells, the lower part of the gastro-intestinal tract and the kidney [2,3,9]. The physiological function of CNP is not known. However, there is evidence which suggests that C-type natriuretic peptides have a pathologic role in cytokine-associated disorders, septic shock and renal failure [10–12]. In particular, the role of CNP as a potentially potent toxin is highlighted by the fact that CNP from platypus venom was shown to be associated with sustained tonic relaxation of the rat uterus in vitro, an effect mimicked by synthetic CNP [8,11]. The molecular mechanisms underlying the action of these peptides are not well known. It is thought that they act principally via the $ANP_B$ receptor, particulate guanylate cyclase-B [6–13], leading to an increase in the level of cGMP [14] which regulates ion transport pathways [15–18]. This in turn results in the inhibition of muscle contraction leading to vasodilation and modification of fluid and electrolyte homeostasis in salt secreting cells [2,8,11,19]. The aims of this study are to examine the ability of synthetic human CNP-22 and the OaCNP-39(18–39) to form ion channels in artificial bilayers and to characterize the type, conductance and kinetic properties of these channels. The data obtained in this study indicate that synthetic human CNP-22 and the OaCNP-39(18–39) may exert their effects by forming ion channels.

## 2. Materials and methods

### 2.1. Synthesis of CNP

CNP-22, 1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21-22 (Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-**Met**-Ser-Gly-Leu-Gly-Cys) and OaCNP-39(18–39), 1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21-22 (Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-**Thr**-Ser-Gly-Leu-Gly-Cys) are routinely synthesized and their amino acid sequences determined at the Center for Molecular Structure and Function at the ANU as detailed previously [8,11].

### 2.2. Lipid bilayer technique

Bilayers were formed across a 150 μm hole in the wall of a 1 ml Delrin cup using a mixture of palmitoyl-oleoyl-phosphatidylethanolamine, palmitoyl-oleoyl-phosphatidylserine and palmitoyl-oleoyl-phosphatidylcholine (5:3:2, by volume) [20,21], obtained in chloroform from Avanti Polar Lipids (Alabaster, AL). The lipid mixture was dried under a stream of $N_2$ and redissolved in *n*-decane at a final concentration of 50 mg/ml. Synthetic CNP-22 or OaCNP-39(18–39), which each have three charged amino acids with a net charge of +2, were then incorporated into the negatively charged lipid bilayer by addition to the *cis* chamber at a final peptide concentration of 0.1–1 μg/ml. The side of the bilayer to which the peptide was added is defined as *cis*, and the other side as *trans*. Ion channels were also recorded from a peptide:lipid mixture of 1:50. Unless it is otherwise stated, the initial experimental solution for incorporating synthetic CNP-22 or OaCNP-39(18–39) into the bilayers contained KCl (250 mM *cis* and 50 mM *trans*) plus 1 mM $CaCl_2$ and 10 mM HEPES buffer (adjusted with 4.8 mM KOH to pH 7.4). Potassium channel blockers (Sigma) stock solutions are added to the *cis* or *trans* chambers. The experiments are conducted at 20–25°C.

### 2.3. Ion channel recording

The pClamp6 program (Axon Instruments) is used for voltage command and acquisition of ionic current families with an Axopatch 200 amplifier (Axon Instruments). The current is monitored with an oscilloscope and the data are stored on a computer. The *cis* and *trans* chambers are connected to the amplifier head stage by Ag/AgCl electrodes in agar, salt bridges containing solutions are present in each chamber. Voltages and currents are expressed relative to the *trans* chamber. Data are filtered at 1 kHz (4-pole Bessel, −3 dB) and digitized via a TL-1 DMA interface (Axon Instruments) at 2 kHz.

### 2.4. Data analysis

Data are obtained only from bilayers having a specific capacitance of $> 0.42$ μF/cm$^2$ and which contain one active $K^+$ channel [21].

*Fax: (61) (2) 6249 0760.
E-mail: joseph.kourie@anu.edu.au


PII: S0014-5793(99)00081-2

Kinetic analysis was conducted only for optimal bilayers that contained a single active channel. Single channel activity is analyzed for overall characteristics using the program Channel 2 [21]. It is used to obtain the open probability, $P_o$, i.e. the fraction of time that the channel is open. The threshold level for the detection of single channel events is set at 50% of the maximum current [22]. The reversal potential ($V_{rev}$) was corrected for ionic mobility and liquid junction potential [23] and the relative ion permeabilities were calculated according to the equation [24]:

$$P_X/P_K = \{[K^+]_{trans} - [K^+]_{cis}\exp(zFV_{rev}/RT)\}/([X^+]_{cis}\exp(zFV_{rev}/RT)).$$

## 3. Results

We used pClamp6 software (Axon instruments) to command and acquire ionic currents through single CNP-22- and OaCNP-39(18–39)-formed channels in lipid bilayer membranes clamped at potentials between −70 and +80 mV. The channel activity is characterized by a tendency for inactivation at negative voltages, e.g. −60 and −70 mV. The constructed current-voltage relationships of the single CNP-22-formed ion channels currents (Fig. 1B), which show weak outward rectification, were fitted with two exponentials. The current's reversal potential ($V_{rev}$) of −23.2 ± 4.7 mV ($n = 3$) was closer to the $E_{K^+}$ (−35 mV) than $E_{Cl^-}$ (+35 mV), indicating that the current was mainly due to the movement of $K^+$, though some $Cl^-$ also passed through the channel.

The time course of the current transitions reveals inactivation of the channel at voltages more negative than −40 mV (Fig. 1C). At −70 mV, 12 out of 16 episodes showed channel inactivation after 1.3 ± 0.4 s ($n = 12$). At −60 mV these values were observed in seven out of 16 episodes and the channel inactivated after 1.7 ± 0.4 s ($n = 7$). The voltage dependence of the inactivation was determined in bilayer membranes containing multiple active CNP-22 (●) and OaCNP-39(18–39) (■) channels by plotting the ratio ($I_{ss}/I_{in}$) of the current at the end of the voltage step to the initial current activated immediately after the voltage step (Fig. 1D). The data indicate that 50% reduction in the inactivation ratio $I_{ss}/I_{in}$ of CNP-22- and OaCNP-39(18–39)-formed channels occurs at −56.7 ± 5.4 mV ($n = 1$–3).

The CNP-22- and OaCNP-22-formed channels were stable and irreversibly associated with the lipid bilayers that maintained their specific capacitance of $> 0.42$ μF/cm$^2$. These findings indicate that it is unlikely that these formed channels were due to peptide-induced structural disturbances of the bilayer. Furthermore, CNP-22 and OaCNP-22, when inactivated by boiling the peptides in 250 mM KCl, failed to form channels in the lipid bilayer at comparable concentrations and for similar durations, indicating that certain natural features of the bioactive peptide are needed for channel formation. In order to ascertain that this was not due to a low probability of peptide incorporation into the lipid bilayer, liposomes [25]

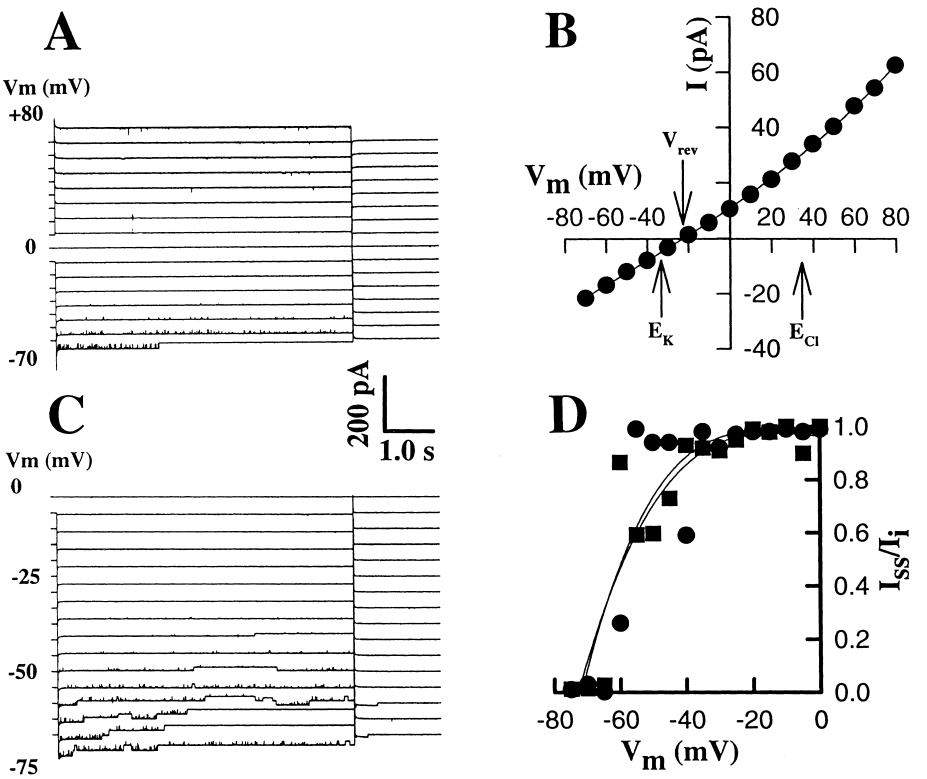


Fig. 1. Voltage dependence of CNP-22-formed single cation channels. A: Representative current traces illustrating single channel activity recorded from a voltage-clamped optimal bilayer, i.e. specific bilayer capacitance $> 42$ μF/cm$^2$ in asymmetrical KCl (250 mM/50 mM, *cis*/*trans*) plus 1 mM $CaCl_2$ and 10 mM HEPES (pH 7.4, adjusted with Tris). Following the convention, the upward deflections denote activation of the outward cation current, i.e. potassium ions moving from the *cis* chamber to the *trans* chamber. For a better display the data are filtered at 1 kHz, digitized at 2 kHz and reduced by a factor 10 and the traces are offset by 10 pA. B: The current-voltage relationship exhibited a weak outward rectification, which is characteristically fitted with two exponentials. C: Current traces illustrating inactivation of the single channel activity at negative voltages. The rest as in A. D: The voltage dependence of the ratio, $I_{ss}/I_{in}$, the current at the end of the voltage step to the initial current activated immediately after the voltage steps. Symbols are for two bilayers, each containing three active CNP-22 (●) and OaCNP-39(18–39) (■) channels. The solid lines are drawn to a third order polynominal fit of the data for channels.

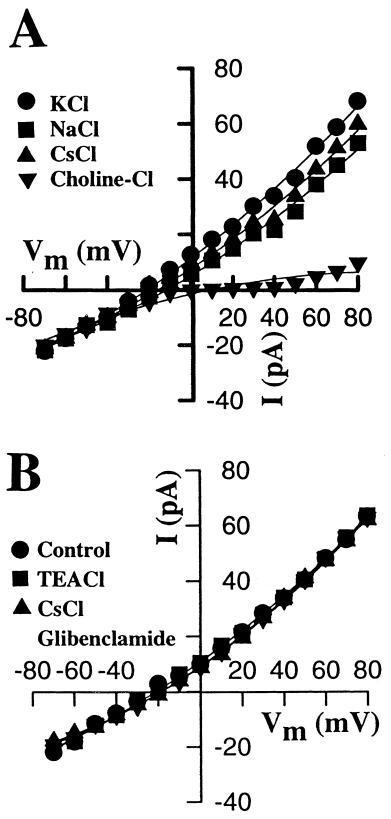


Fig. 2. Ion selectivity of the OaCNP-39(18–39)-formed channel. A: Current-voltage relationships for different cations: (●) $K^+$, (■) $Na^+$, (▲) $Cs^+$, and (▼) choline$^+$. B: Effects of $K^+$ channel blockers. Current voltage relationships (●) control, (■) 20 mM $TEA^+_{cis}$, (▲) 20 mM $Cs^+_{cis}$, and (▼) 0.5 mM glibenclamide$_{cis}$.

were used to detect the incorporation of the peptide into the bilayer, by monitoring the specific capacitance of the lipid bilayer. Although the liposomes were incorporated into the bilayer as indicated by the increase in the specific capacitance, no channel activities were observed for either CNP-22 or OaCNP-22 ($n = 4$ and 5), respectively.

We further determined the cation selectivity of the channel in ion substitution experiments. The 250 mM KCl in the *cis* chamber was totally replaced by 250 mM choline-Cl, NaCl, or CsCl and families of current traces were obtained at voltages between −70 and +80 mV. During these replacements the channel protein remained irreversibly associated with the lipid bilayer. The constructed current-voltage relationships from these current families revealed reduction in the current amplitude and only small shifts in $V_{rev}$, indicating a poor monovalent cationic selectivity (Fig. 2A). The sequence and the mean permeability ratios for three channels (one CNP-22 and two OaCNP-39(18–39)) were found to be $P^+_K : P^+_{Cs} : P^+_{Na} : P^+_{choline}$ 1:0.88:0.76:0.13, respectively.

We also examined the pharmacology of the channel by determining its sensitivity to $K^+$ channel antagonists. We found that this cation channel is neither a $TEA^+$-sensitive, a $Cs^+$-sensitive nor an ATP-sensitive $K^+$ channel, since neither the addition of 50 mM $TEA^+_{cis}$ (a blocker of the outwardly rectifying $K^+$ channel), 20 mM $Cs^+_{cis}$ (a blocker of the inwardly rectifying $K^+$ channel) nor 0.5 mM glibenclamide$_{cis}$ (a blocker of the ATP-sensitive $K^+$ channel) to the *cis* chamber affect the time course or the kinetics of the OaCNP-39(18–39)-formed channels. Neither the weak outward rectification of the current-voltage relations nor the $V_{rev}$ were affected by these $K^+$ channel blockers (Fig. 2B).

We obtained the kinetic parameters of the CNP-22 and OaCNP-39(18–39)-formed channels by analyzing channel activity recorded at positive and negative voltages (Fig. 3). At positive voltages between 0 and +80 mV, the probability of the channel being open, $P_o$, was virtually constant and had a value of 1. At negative voltages between −70 and −10 mV, the $P_o$ had a bell-shaped curve with a peak at −60 mV. The channel frequency, $F_o$, decreased from 14.96 events/s at −70 mV to 0.8 events/s at +80 mV. The mean open time, $T_o$, increased from 65 ms at −70 mV to 201 ms at −50 mV and to 335 ms at −30 mV. In contrast, the mean closed time, $T_c$, decreased exponentially from 16.4 ms at −70 mV to 0.5 ms at −20 mV.

The decline in the probability of the channel being open at voltages more negative than −70 mV is due to the inactivation of the channel ($n = 23$ families of current traces from three channels). Note that in all current traces the CNP-22- or OaCNP-39(18–39)-formed channels became inactivated at negative voltages. For example, in Fig. 1C, the bilayer which contained at least three channels shows such a channel inactivation at negative voltages. To confirm that the decline in the open probability of the channel at negative voltages is due to channel inactivation, we constructed the ensemble average of the currents of 20 episodes at −50, −60, −70 and −80 mV (Fig. 4). The data show that the channel was open at −50 and −60 mV. On the other hand, at −70 and −80 mV it tended to inactivate and the inactivation of the currents could be described by two exponentials, where τ1 and τ2 are, respectively, 537 ms and 952 ms at −70 mV and 719 and 2937 ms at −80 mV. These findings indicate that the decline in the open probability at $V_m$ is more negative than −70 mV, because of channel inactivation.

## 4. Discussion

The channel activity reported here was seen only when peptides were added to the *cis* chamber, and thus points to their interaction with the lipid bilayers. The data in Figs. 1–4 indicate that the conductance and kinetic properties of the cation channel were not affected by substitution of Met (CNP-22) to Thr (OaCNP-39(18–39)) at the amino acid position 17 (see Section 2) ($n = 32$). Similarly, the respective compositions of the phospholipid mixture, 5:3:2 PE:PS:PC and 5:5 PE:PS, had no effects on the channel activity ($n = 5$). The fact that OaCNP-39(18–39) forms channels in lipid bilayer membranes at pH 7.4 and voltages between −80 and +80 mV suggests that voltage and acidic conditions are not required for the peptide domains to dock onto and interact with the bilayer in order to form ion channels, as suggested for colicin E1 [26] and Bcl-2-formed channels [27]. These findings, taken together with the biophysical properties of the 546 pS channel, point to a channel that is different from the previously reported peptide- or toxin-formed voltage-dependent cation channels such as the staphylococcal δ-toxin-induced 70–100 and 450 pS channels [28], the tetanus toxin-induced 89 pS channel (500 mM KCl) [29] and the *Clostridium botulinum* C2-II toxin-induced 55 pS channel (100 mM KCl) [30]. This weakly cation-selective channel may represent a new member of the class of amyloid forming peptides (Table 1) which form channels and

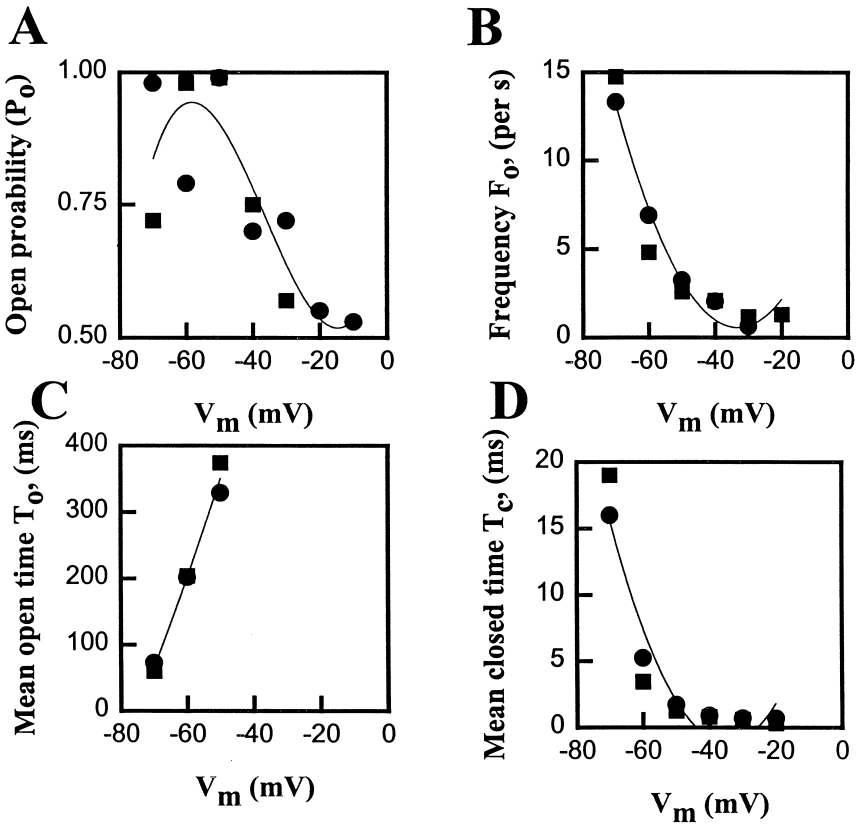


Fig. 3. Voltage dependence of the CNP-22- (●) and OaCNP-39(18–39)- (■) formed channel kinetic parameters. A: Open probability ($P_o$). B: Frequency $F_o$. C: Mean open time $T_o$. D: Mean closed time $T_c$. The solid lines are drawn to a third order polynomial fit in A, B and D and to a second order polynomial fit in C.

it may be responsible for disease pathology in various amyloidoses. This class of peptides, whose channel forming characteristics are shown in Table 1, includes: AβP-formed $Ca^{2+}$ channel [25,31,32], prion peptide PrP (106–126)-formed voltage-independent 20–60 pS (in 100 mM NaCl) non-selective (PNa/PCl ~2.5) ion channel [33] and amylin-formed voltage-dependent 7.5 pS (in 10 mM KCl) non-selective ion-permeable channel [34]. Although natriuretic peptides differ from amyloids in their amino acid sequence, charged domains and

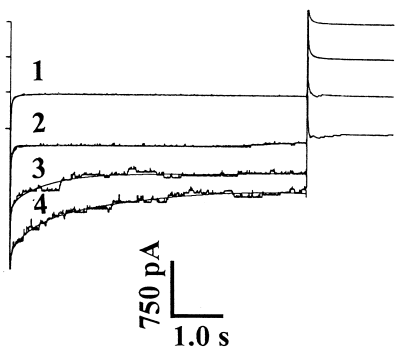


Fig. 4. The mean ($n=21$) ensemble current through the OaCNP-39(18–39)-formed channel clamped to 1 = −50, 2 = −60, 3 = −70 and 4 = −80 mV from a holding potential of 0 mV. After the initial large transient capacitive current, the channels are activated and remain open in 1 (see also Fig. 1C) and are inactivated in 2, 3 and 4. The solid lines are drawn to a second order polynomial fit in 3 and 4. For a better display, the ensemble current traces are offset by 10 pA.

the disulfide-linked loop (Table 1), there appear to be some findings to suggest that natriuretic peptides may be related to amyloids. These findings include: (a) common motifs, which make both types of peptides substrates to the insulin-degrading enzyme [35] and the GLG motif: (b) atrial natriuretic peptide is a precursor or a major subunit of amyloid [36,37]: (c) natriuretic peptides and amyloids elevate cGMP via interaction with guanylate cyclase receptors [38–40]: (d) natriuretic peptides are occurring together with amyloid in amyloid deposits [41]. The prion peptide PrP (106–126) also does not have a disulfide-linked loop, the GlG motif or the charges in the center of its amino acid sequence.

The structure of the natriuretic peptides (ANP, BNP and CNP) incorporates a 17 residue intra-molecular disulfide loop (between residues 6 and 22) with amino acid sequences which are identical in ANP and BNP, whilst CNP is the same, except for two residues [1–4,9]. In contrast to ANP and BNP, which possess C- and N-terminal sequence extensions beyond this loop, CNP extends only N-terminally [6,8,11]. Therefore, it is not unreasonable to suggest that ANP and BNP may also form ion channels.

This study provides evidence showing that, in addition to CNP22-activated guanylate cyclase [14] which induces cGMP release that interacts with $K^+$ channels [15,16], $Cl^-$ channels sodium absorption in murine airway epithelium [17,18] and chloride secretion in the rectal gland of *Squalus acanthias* [43], CNP-22 may also function, by virtue of its ability to form ion channels, as an efficient ion transport pathway for signal transduction. The concentrations of CNP-22 or

Table 1
Properties of the class of amyloid-forming peptides (amylins, prions, AβPs and CNPs) which form channels that may be responsible for disease pathology in various amyloidoses

| Peptide type | Channel type and selectivity | Method | Concentration of *cis*/*trans* (mM) | Conductance (pS), *I-V* relations, blockers | Amino acid sequence and mechanism of channel formation | Pathology | Reference |
|---|---|---|---|---|---|---|---|
| Human amylin, 37 amino acids (net charge +5) hormone co-secreted with insulin from the pancreatic β cells | Non-selective | 1–10 μM peptide incorporated into lipid bilayer azolectin phosphotadylcholine, GPA | 10/10 KCl | 7.5, linear | KCNTATC ATQRLANFLV HSSNNFGAIL SSTNVGSNTY<br><br>Amylin interacts with PC and gets transformed from a combination of α-helical and β-sheet structures to largely β structures-formed channels [42] | Type II diabetes mellitus | [34] |
| Prion protein (PrP) fragment, 21 amino acids, net charge +3 | $Ca^{2+} > Na^+ > K^+ > Li^+ > Rb^+ > Cs^+ > Cl^-$ | 0.1–20 μM age (9 days) and low pH (4.5–5): enhances channel incorporation into lipid bilayer; azolectin phosphatidylcholine, GPA | 100/100 NaCl | 20, 40, 60, 100, 120, linear | KTNMKHMAGAAAAGAV-VGGLG<br><br>Prion protein (106–126) in the form of a β-sheet structure forms non-selective channels. Leakage-induced loss of ionic homeostasis mediated apoptosis | Neurodegenrative diseases e.g. scrapie, encephalopathies in animals and Kuru and Creutzfeldt-Jacobs disease in humans | [33] |
| AβP (1–40) amino acids | Cation selective. $Ca^{2+}$ channel PK/PCl=11. $Cs^+ > Li^+ > Ca^{2+} \geq K^+ > Na^+$ | 5 μg PS liposomes fused into PE:PS bilayer | 40/40 KCl, 40/60 KCl | 325, 346, linear<br><br>Blocked reversibly with mM tromethamine. Blocked irreversibly μM $Al^{3+}$ | DAEFRHDSGYEV HHQKLVFFA EDVGSNKGAII GLMVGGVV<br><br>Forming dimers, trimers and tetramers (combination of α-helical and β-sheet structures to largely β) | Alzheimer's disease | [25] |
| AβP (1–40) amino acid | $Ca^{2+}$ channel | 5 μg PS liposomes fused into PE:PS bilayer | 40/40 KCl | μm $Zn^{2+}$ blocks conductances $< 400$ mM $Zn^{2+}$ blocks conductances $> 400$ | $Zn^{2+}$ binding to the large cation forming channels is viewed as protective of amyloid neurotoxicity | Alzheimer's disease | [32] |
| Synthetic human CNP-22 and OaCNP-39(18–39), 22 amino acids, net charge +2 | Weak cation-selective channel $K^+ > Cs^+ > Na^+ > >$ $Choline^+$ | 0.1–1 μg/ml PE:PS:PC 50:30:20 | 250/500 KCl | 546, weak outward rectification, insensitive to 20 mM TEACl, 20 mM CsCl and 0.5 mM glibenclamide | GLSKGCFGLKLDRIGSTSGLGC<br><br>Oligomeric complexes, large cation forming channels | Vasodilation, changes in electrolyte homeostasis, kidney failure, envenomation [8,11] | this study |

OaCNP-22 used to obtain channel activity are comparable to concentrations of several cytotoxic channel forming peptides (see [34]). In vivo the CNP-22-formed weakly cation-selective 546 pS channel with high open probability is expected to induce changes in $V_m$, second messenger systems (e.g. cGMP and $Ca^{2+}$ homeostasis) and electrolyte homeostasis.

*Acknowledgements:* I would like to thank Drs. G. de Plater and P. Milburn for their encouragement and generous gift of natriuretic peptides. I also thank Dr. S.-H. Chung and Mr. R. McCart for numerous discussions, suggestions and critical reading of the manuscript. The laboratory assistance of Ms. A. Culverson, C. Horan and E. Sturgiss of the CSIRO Research Scheme is greatly appreciated. The referees are also acknowledged for their constructive suggestions. This research work is supported by a National Health and Medical Research Council project Grant (970122) and Australian Research Council Small Research Grant (F99123).

## References

[1] Kojima, M., Minamino, N., Kangawa, K. and Matsuo, H. (1990) FEBS Lett. 276, 209–213.
[2] Sudoh, T., Minamino, N., Kenji, K. and Matsuo, H. (1990) Biochem. Biophys. Res. Commun. 168, 863–870.
[3] Komatsu, Y., Nakao, K., Suga, S., Ogawa, Y., Mukoyama, M., Arai, H., Shirakami, G., Hosoda, K., Nakagawa, O., Hama, N., Kishimoto, I. and Imura, H. (1991) Endocrinology 129, 1104–1106.
[4] Mukoyama, M., Nakao, K., Hosoda, K., Suga, S., Saito, Y., Ogawa, Y., Shirakami, G., Jougasaki, M., Obata, K., Yasue, H., Kambayashi, K., Inouye, K. and Imura, H. (1991) J. Clin. Invest. 87, 1402–1412.
[5] Nakao, K., Ogawa, Y., Suga, S. and Imura, H. (1992) J. Hypertens. 10, 907–912.
[6] Suga, S., Nakao, K., Hosoda, K., Mukoyama, M., Ogawa, Y., Shirakami, G., Arai, H., Saito, Y., Kambayashi, Y., Inouye, K. and Imura, H. (1992) Endocrinology 130, 229–239.
[7] Hama, N., Itoh, H., Shirakami, G., Suga, S., Komatsu, Y., Yoshimasa, T., Tanaka, I., Mori, K. and Nakao, K. (1994) Biochem. Biophys. Res. Commun. 198, 1177–1182.
[8] de Plater, G., Martin, R.L. and Milburn, P.J. (1995) Toxicon 33, 157–169.
[9] Suga, S., Itoh, H., Komatsu, Y., Ogawa, Y., Hama, N., Yoshimasa, T. and Nakao, K. (1993) Endocrinology 133, 3038–3041.
[10] Murayama, N., Hayashi, M., Ohi, H., Ferreira, L.A.F., Hermann, V., Saito, H., Fujita, Y., Higuchi, S., Fernandes, B.L., Yamane, T. and de Camargo, A.C.M. (1997) Proc. Natl. Acad. Sci. USA 94, 1189–1193.
[11] de Plater, G.M., Martin, R.L. and Milburn, P.J. (1998) Toxicon 36, 847–857.
[12] Suga, S., Nakao, K., Itoh, H., Komatsu, Y., Ogawa, Y., Hama, N. and Imura, H. (1992) J. Clin. Invest. 90, 1145–1149.
[13] Koller, K.J., Lowe, D.G., Bennett, G.L., Minamino, W., Kangawa, K., Matsuo, H. and Goeddel, D.V. (1991) Science 252, 120–123.
[14] Stingo, A.J., Clavell, A.L., Arthus, L. and Burnett Jr., J.C. (1992) Am. J. Physiol. 262, H308–H312.
[15] White, R.E., Lee, A.B., Shcherbatko, A.D., Lincoln, T.M., Schonbrunn, A. and Armstrong, D.L. (1993) Nature 361, 263–266.
[16] Wei, C.M., Hu, S., Miller, V.M. and Burnett, J.C.J. (1994) Biochem. Biophys. Res. Commun. 205, 765–771.
[17] Kelley, T.J., Cotton, C.U. and Drumm, M.L. (1997) Am. J. Physiol. 273, L1065–L1072.
[18] Kelley, T.J., Cotton, C.U. and Drumm, M.L. (1998) Am. J. Physiol. 273, L990–L996.
[19] Morita, H., Hagike, M., Horiba, T., Miyake, K., Ohyama, H., Yamaouchi, H., Hosomi, H., Kangawa, K., Minamino, N. and Matsuo, H. (1992) Jpn. J. Physiol. 42, 349–353.
[20] Miller, C. and Racker, E. (1978) J. Membr. Biol. 30, 283–300.
[21] Kourie, J.I., Laver, D.R., Junankar, P.R., Gage, P.W. and Dulhunty, A.F. (1996) Biophys. J. 70, 221.
[22] Patlak, J.B. (1993) Biophys. J. 65, 29–42.
[23] Barry, P.H. (1994) J. Neurosci. Methods 51, 107–116.
[24] Hodgkin, A.L. and Katz, B. (1949) J. Physiol. 108, 37–77.
[25] Arispe, N., Rojas, E. and Pollard, H.B. (1993) Proc. Natl. Acad. Sci. USA 90, 567–571.
[26] Merrill, A.R., Steer, B.A., Prentice, G.A., Weller, M.J. and Szabo, A.G. (1997) Biochemistry 36, 6874–6884.
[27] Minn, A.J., Velez, P., Schendel, S.L., Liang, H., Muchmore, S.W., Fesik, S.W., Fill, U. and Thompson, C.B. (1997) Nature 385, 353–357.
[28] Mellor, I.R., Thomas, D.H. and Sansom, M.S. (1988) Biochim. Biophys. Acta 942, 280–294.
[29] Gambale, F. and Montal, M. (1988) Biophys. J. 53, 771–783.
[30] Schmid, A., Benz, R., Just, I. and Aktories, K. (1994) J. Biol. Chem. 269, 16706–16711.
[31] Arispe, N., Pollard, H.B. and Rojas, E. (1993) Proc. Natl. Acad. Sci. USA 90, 10573–10577.
[32] Arispe, N., Pollard, H.B. and Rojas, E. (1996) Proc. Natl. Acad. Sci. USA 93, 1710–1715.
[33] Lin, M.-C., Mirzabekov, T. and Kagan, B.L. (1997) J. Biol. Chem. 272, 44–47.
[34] Mirzabeckov, T.A., Lin, M.C. and Kagan, B.L. (1996) J. Biol. Chem. 271, 1988–1992.
[35] Kurochkin, I.V. (1998) FEBS Lett. 427, 153–156.
[36] Takemura, G., Takatsu, Y., Doyama, K., Itoh, H., Saito, Y., Koshiji, M., Ando, F., Fujiwara, T., Nakao, K. and Fujiwara, H. (1998) J. Am. Coll. Cardiol. 31, 754–765.
[37] Takahashi, M., Hoshii, Y., Kawano, H., Gondo, T., Yokota, T., Okabyashi, H., Shimada, I. and Ishihara, T. (1998) Int. J. Exp. Clin. Invest. 5, 35–42.
[38] Barger, S.W. and Mattson, M.P. (1995) Biochem. J. 311, 45–47.
[39] Teng, M.H. and Buxbaum, J.N. (1996) Int. J. Exp. Clin. Invest. 3, 187–208.
[40] Jan, L.Y. and Jan, Y.H. (1997) Curr. Opin. Cell Biol. 9, 155–160.
[41] Westermark, P. (1997) Int. J. Exp. Clin. Invest. 1, 47–60.
[42] Mclean, L.R. and Balasubramaniam, A. (1992) Biochem. Biophys. Acta 1122, 317–320.
[43] Solomon, R., Protter, A., McEnroe, G., Porter, J.G. and Silva, P. (1992) Am. J. Physiol. 262, R707–R771.