Effect of hypoosmolality on the abundance, poly(A) tail length and axonal targeting of arginine vasopressin and oxytocin mRNAs in rat hypothalamic magnocellular neurons

Pernille C. Svane[a], Niels A. Thorn[a,*], Dietmar Richter[b], Evita Mohr[b]

[a]*Danish Biotechnology Centre for Signal Peptide Research, Department of Medical Physiology, University of Copenhagen, The Panum Institute Building 12.2., Blegdamsvej 3, DK-2200 Copenhagen N, Denmark*
[b]*Institut für Zellbiochemie und klinische Neurobiologie, Universität Hamburg, Universitätskrankenhaus Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany*

Received 9 August 1995

Abstract Arginine vasopressin (AVP) and oxytocin (OT) mRNAs are targeted to the axonal compartment of rat hypothalamic magnocellular neurons. Salt-loading results in a considerable rise in hypothalamic and axonal AVP mRNA but only a moderate increase for axonal OT mRNA. Here we report that hypoosmolality gives rise to a rapid decrease of axonal AVP encoding transcripts to undetectable levels after 2 weeks. The levels of OT mRNA in the axonal compartment did not change significantly. In the hypothalamus the mRNA for AVP also decreased. The size of the poly(A) tract of AVP encoding transcripts appeared to be strictly correlated with plasma osmolality. In contrast, the amount and size of OT encoding mRNAs were only moderately or not influenced by hypoosmolar stimuli.

Key words: Arginine vasopressin; Oxytocin; Messenger RNA; Hypoosmolality; Poly(A) tail size; Axonal mRNAs

1. Introduction

The neurohypophyseal hormones arginine vasopressin (AVP) and oxytocin (OT) are synthesized in hypothalamic magnocellular neurons of the nucleus supraopticus (SON) and the nucleus paraventricularis (PVN) (for review see [1]). In the rat, the rate of synthesis and release of AVP and, surprisingly, that of OT rises during conditions of elevated plasma osmolality (for review see [2]) and is apparently regulated at the level of gene expression [3]. Hyperosmolality induced by either salt-loading or water deprivation has been shown to result in a two-to three-fold increase in the abundance of AVP as well as OT encoding mRNAs (for review see [2]). Interestingly, this is accompanied by an elongation of their poly(A) tails although the physiological significance remains to be determined [4–6]. Both AVP and OT mRNAs are subject to axonal targeting [7–9] and hyperosmolar stimuli give rise to a differential increase in the level of AVP (17-fold) and OT (3-fold) mRNAs in the axonal compartment [8,10–12]. The axonal transcripts exhibit shorter poly(A) tracts than their counterparts in the cell bodies [2], and a poly(A) tail polymorphism following osmotic challenge is not observed for these transcripts [12]. In the present experiments we have attempted to determine the intracellular distribution of the peptide hormone mRNAs in response to a hypoosmolar stimulus. This was achieved by constant systemic infusion of the AVP V2-receptor agonist 1-deamino-[8-D-arginine] vasopressin (DDAVP) by use of subcutaneously implanted osmotic minipumps combined with a liquid diet to induce overhydration. In the hypothalamus hypoosmolality induced a reduction in both the abundance of AVP mRNA and the length of its poly(A) tract; the latter appears to be strictly correlated with plasma osmolality. In addition, a rapid and steady depletion of the axonal AVP mRNA pool was observed with undetectable levels after 2 weeks of overhydration. Surprisingly, the oxytocinergic system was considerably less affected by this treatment.

2. Materials and methods

2.1. Overhydration protocol

Male Wistar rats (190–300 g, The Panum Institute, Copenhagen, Denmark) were individually housed in normal plastic rat cages under standard housing conditions (14 h light cycle, lights on 6.00 h, off 20.00 h; temperature 21°C).

We used a slight modification of a previously published procedure [13] to induce plasma hypoosmolality. Three days before pump implantation and on the first day after pump implantation the rats were fed 65 g of a complete, dilute liquid diet (76.2% (w/w) water, 1.03 kcal/g). Day 1 after pump implantation and the following days the rats were fed 50 g of a more concentrated diet (63.9% (w/w) water, 1.71 kcal/g). The diet was supplemented with sodium chloride up to 5 g/kg food to fulfill the rats' need for sodium [14]. The days before pump implantation the rats had free access to drinking water. After pump implantation the water bottles were removed from rats submitted to overhydration. Each experimental and control group contained three rats and the experiment was repeated three times. The control groups were either non-treated rats given food and water ad libitum for 14 days, non-treated rats decapitated on the day of delivery from the animal house or rats implanted with a pump containing only vehicle (10 mM acetic acid, pH 5.5) and fed liquid diet as the overhydrated animals, but with free access to drinking water throughout the experiment. Alzet osmotic minipumps model 2002 containing DDAVP (1-deamino-[8-D-arginine] vasopressin) in 10 mM acetic acid, pH 5.5, corresponding to the rats receiving 2 ng/h of DDAVP, were implanted subcutaneosly between the scapulae under anaesthesia. The experimental animals showed continous weight gain after the surgery. The weight gain on the day of decapitation relative to the day of pump implantation for vehicle treated rats did not differ significantly from that of overhydrated rats after 14 days (23% ± 6, (n = 2) and 21% ± 1, (n = 3), mean ± S.E.M., number of independent experiments in brackets, data analyzed by Student's t-test, two-tailed).

2.2. Preparation of RNA, removal of poly(A) tails and Northern blot hybridization

Before decapitation rats were anaesthesized. The brain was removed and the SON and PVN were dissected by using a modified Jacobowitz slicer (RBM-4000C, Activational Systems Inc., USA) The neurointermediate lobes (NILs) containing the axons of hypothalamic magnocel-

*Corresponding author. Fax: (45) 35 32 75 37.

SSDI 0014-5793(95)01008-4

lular neurons were removed under a stereomicroscope. RNAs from these tissues were prepared by using RNAzol B according to the instructions of the manufacturer. To allow for evaluation of RNA recovery in different samples, a constant amount of in vitro transcribed isotocin sense cRNA derived from a cDNA clone encoding the teleost fish *Catostomus commersoni* isotocin (IT) precursor [15] was added to each of the RNA homogenates prior to chloroform extraction and alcohol precipitation. RNAs were subsequently resolved by agarose gel electrophoresis following glyoxylation and transferred to nylon membranes according to standard procedures [16]. Northern blot hybridizations with ^{32}P-labelled probes specific for rat AVP and OT as well as fish IT mRNAs were performed as described [2]. Removal of the poly(A) tail was done as described [12].

2.3. Analysis of plasma osmolality

Trunk blood was collected in chilled, heparinized tubes and placed on ice until centrifugation at $1,500 \times g$ at 4°C for 10 min. Plasma samples were kept at 4°C until measurements of osmolality by freezing point depression (The AdvancedTM Osmometer 3D3, USA). In one experiment plasma sodium concentration was measured in parallel to plasma osmolality by flame spectrophotometry and found to be directly proportional to plasma osmolality (data not shown).

2.4. Materials and drugs

The diet used in the overhydration protocol (Complan) was purchased from Woekl, distributed by MEDA A/S, Herlev, Denmark. Alzet osmotic minipumps (model 2002) were from Alza corporation, Palo Alto, Ca., USA. DDAVP was purchased from Ferring, Malmö, Sweden. RNAzol B was purchased from Angewandte Gentechnologie Systeme, Heidelberg, Germany.

3. Results

3.1. Plasma osmolality in response to systemic DDAVP administration

Overhydration of rats induced by systemic administration of 2 ng/h of DDAVP by osmotic minipumps in combination with a liquid diet induced a marked decrease in plasma osmolality (Fig. 1). All animals that had been subjected to the overhydration regimen showed significantly ($P_{\text{One-tailed}} < 0.05$) lower

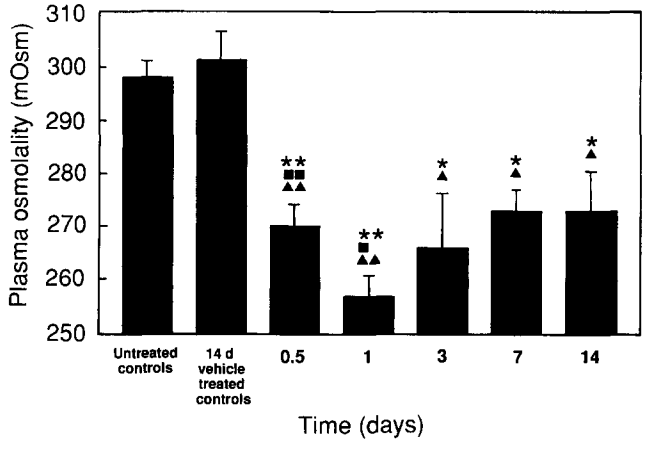

Fig. 1. Plasma osmolality in rats made hypoosmolar with DDAVP and liquid diet (day 0.5 to day 14). Control rats were either untreated rats or rats implanted with vehicle filled pumps and receiving a liquid diet for 14 days. Data are given as means ± S.E.M. of 3 independent experiments, each including 3 rats, except for controls where the number of independent experiments were 2. * and **: $P_{\text{One-tailed}}$ respectively < 0.005 and < 0.001 relative to vehicle treated controls, ▲ and ▲▲: $P_{\text{One-tailed}}$ respectively < 0.005 and < 0.001 relative to untreated controls, ■ and ■■: $P_{\text{One-tailed}}$ respectively < 0.005 and < 0.001 relative to the day before, using Student's *t*-test. There was no significant difference between the plasma osmolalities of untreated control rats and control rats treated with vehicle for 14 days.

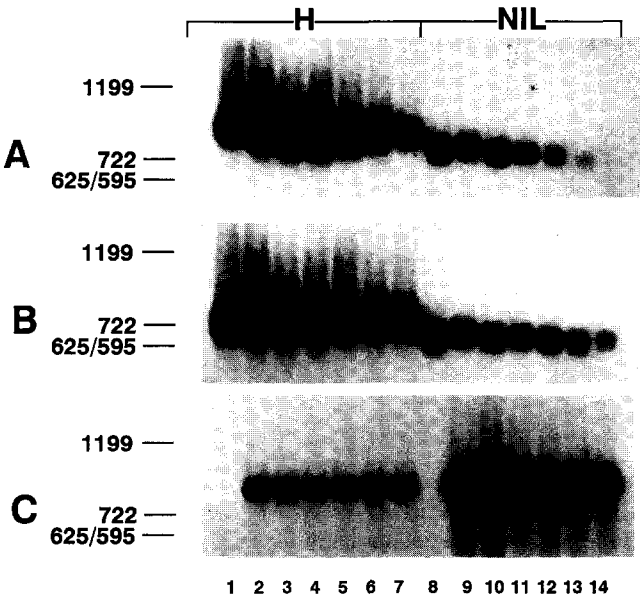

Fig. 2. Northern blot analyses of RNAs extracted from hypothalami (H) (lanes 1–7) and neurointermediate lobes (NIL) (lanes 8–14) of rats subjected to overhydration by systemic administration of the arginine vasopressin (AVP) V2 receptor agonist 1deamino-[8-D-arginine] vasopressin: lanes 1 and 8, untreated controls; lanes 2 and 9; control rats receiving vehicle and liquid diet for 14 days; lanes 3 and 10, 12 hours of overhydration; lanes 4 and 11, 24 hours of overhydration; lanes 5 and 12, 3 days of overhydration; lanes 6 and 13, 7 days of overhydration; lanes 7 and 14, 14 days of overhydration. A. The membrane was first hybridized with a ^{32}P-labelled AVP-specific probe. B. After removal of the hybridized probe the membrane was subsequently hybridized with a ^{32}P-labelled oxytocin (OT)-specific probe. C. Hybridized material was again washed off and the filter was finally hybridized with a ^{32}P-labelled probe specific for *Catostomus commersoni* isotocin (IT) RNA. It can be seen that the signal intensities in lanes containing hypothalamic or NIL RNAs, respectively, do not vary considerably, indicating similar efficiencies in RNA recovery from these tissues. Note that no IT cRNA was added to RNA extracted from untreated control animals (lanes1 and 8). DNA size markers in bases are indicated on the left.

plasma osmolality values when compared to either untreated or vehicle treated control groups. The lowest values were consistently observed after 24 h of overhydration. Subsequently, plasma osmolality increased until day 7 to a level which was maintained for the duration of the experiment. The moderate increase in plasma osmolality following overhydration for more than 24 h was not due to pump faults, since liquid delivery was controlled by measurements of pump contents before and after the experiments. Furthermore, the effect was reproducible in independently performed experiments. We suggest that the observed changes in plasma osmolality might be due to changes in the water content of the administered liquid diet (as noted in section 2) or perhaps be the result of receptor desensitization mechanisms.

3.2. Levels of AVP mRNA during hypoosmolality

Northern blot hybridization analyses revealed that, during the 14 days course of overhydration, AVP mRNA levels clearly decreased in the hypothalamus (Fig. 2A). This was accompanied by a reduction in the size of the corresponding transcripts. As revealed by Northern blot hybridization after enzymatical removal of the poly(A) tail by RNase H in the presence of

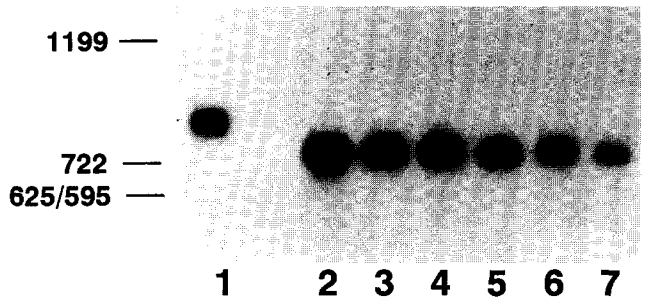

Fig. 3. Northern blot analyses of RNAs extracted from rat hypothalami before (lane 1) and following enzymatical removal of the poly(A) tails (lanes 2–7). The rats have been subjected to overhydration by systemic administration of the arginine vasopressin (AVP) V2 receptor agonist DDAVP: lanes 1 and 2, control rats receiving vehicle and liquid diet for 14 days; lane 3, 12 hours of overhydration; lane 4, 24 hours of overhydration; lane 5, 3 days of overhydration; lane 6, 7 days of overhydration; lane 7, 14 days of overhydration. The membrane was hybridized with a ^{32}P-labelled AVP-specific probe. DNA size markers are indicated on the left.

oligo(dT) the reduced size is due to variation of the poly(A) tail lengths (Fig. 3). A poly(A) tail length polymorphism has previously been described to occur in response to osmotic challenge by Carrazana et al. [4] who found that an elevated plasma osmolality results in an increase in poly(A) tail size. Strikingly, the size of AVP transcripts during overhydration appears to be strictly correlated with plasma osmolality. Compared to the control group, the size of AVP mRNA decreased until a minimal length was observed 24 h following the onset of overhydration. Subsequently, poly(A) tail length increased until day 7, and this size was maintained until the end of the experiment (Fig. 4A). This appears be related to the plasma osmolality values which reached a plateau from day 7 onward (see Fig. 1). The decrease in AVP mRNA levels was even more pronounced with respect to the axonally localized transcripts in the NIL. The levels were already diminished after 24 h of overhydration, and AVP mRNA was barely detectable after 7 days and virtually undetectable after 2 weeks of overhydration. Although minor size differences of the axonal transcripts are apparent on the Northern blot shown in Fig. 2A, these changes were not statistically significant. (Fig. 4A).

3.3. Levels of OT mRNA during hypoosmolality

The levels of hypothalamic OT transcripts were apparently not or only moderately influenced by hypoosmolality. Neither the amount of OT mRNA nor its size varied significantly (Fig. 2B, lanes 1–7). The axonal OT mRNA content, on the other hand, was diminished after 14 days of overhydration (Fig. 2B, lanes 8–14). However, this decrease was not as pronounced as that for axonal AVP-encoding transcripts. No significant size changes were observed during the course of the experiment (Fig. 4B).

4. Discussion

It has been shown previously that hypoosmolality induced by systemic administration of DDAVP combined with a liquid diet results in depletion of AVP mRNA in hypothalamic magnocellular neurons [17]. Data presented here confirm these findings and demonstrate, furthermore, that overhydration does not only affect AVP mRNA levels but also the length of the poly(A) tails which are shorter than those of untreated or vehicle treated control animals. Poly(A) tail shortening of AVP mRNA has also been demonstrated to occur in response to starvation [18]. However, during the course of experiments presented here the animals gained weight throughout the overhydration period and did so at the same rate as rats receiving vehicle and liquid diet. The physiological significance of this poly(A) tail polymorphism is still unclear. It is, however, speculated that transcripts exhibiting longer poly(A) tracts are more efficiently translated compared to those bearing shorter tails [19,20]. If true, AVP precursor synthesis is not only regulated at the transcriptional level by reducing the amount of the corresponding mRNA but also at the level of protein biosynthesis by lowering the translational efficiency. The oxytocinergic system, on the other hand, appears to be hardly affected by hypoosmolar stimuli, since no significant changes in the amount or the size of hypothalamic OT mRNA were observed. An influence of plasma osmolality on the amount and the size of hypothalamic AVP and OT mRNAs has also been shown to

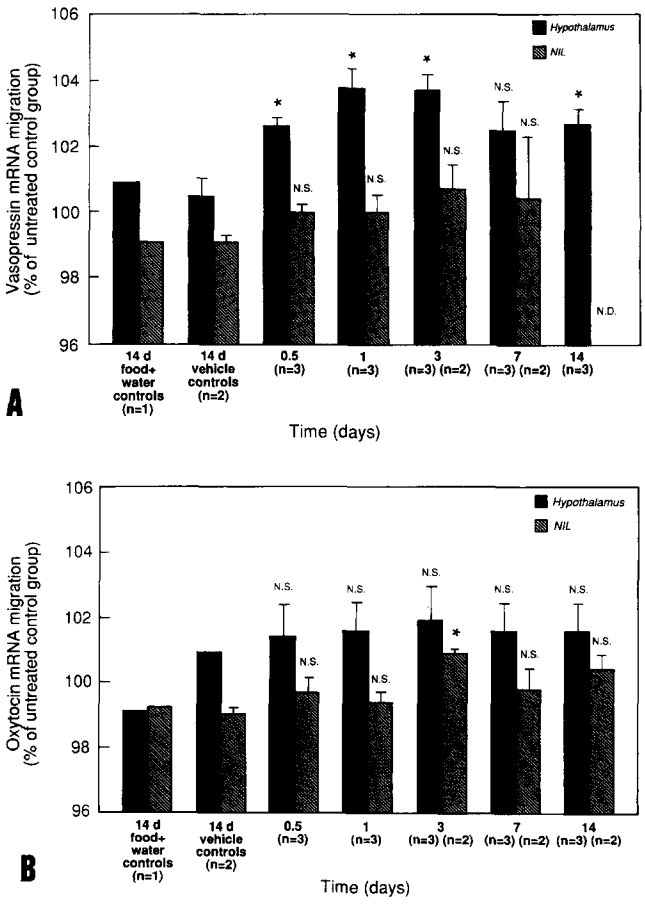

Fig. 4. Effect of hypoosmolality on (A) vasopressin (AVP) mRNA size and (B) oxytocin (OT) mRNA size as determined by Northern analysis of total RNA extracted from hypothalamus and neurointermediate lobe (NIL) as in Fig. 2. The sizes are given as the migration of mRNA in the single groups normalized to the migration of mRNA from untreated controls on the Northern blot (the smaller the mRNA, the longer the distance migrated). Data are given as means ± S.E.M., the number of independent experiments shown in brackets. *: $P_{\text{Two-tailed}} < 0.05$ compared with controls having vehicle filled pumps and fed on liquid for 14 days, N.S.: not significantly different from vehicle treated controls, using Student's t-test, N.D.: signal not detectable on the Northern blot.

occur in response to hyperosmolality [5]. However, in this case the response of vasopressinergic and oxytocinergic cells is very similar in that both peptide hormone-encoding mRNA levels are elevated two- to three-fold after six days of salt loading [6] and both transcript species contain considerably longer poly(A) tracts when compared to those of control animals [4].

Hypoosmolality was accompanied by a progressive reduction of AVP mRNA levels not only in the cell bodies but, even more pronounced, in the axonal domain. After 2 weeks of overhydration, the axonal AVP mRNA content was reduced to undetectable levels. Again, the axonal OT mRNA content was much less affected by hypoosmolality, although a reduction was apparent after 2 weeks of DDAVP administration. A differential accumulation of AVP and OT transcripts in the axonal compartment has also been observed in response to salt loading for seven days which induced a 17-fold increase in the abundance of AVP-encoding transcripts while OT mRNA levels were only moderately increased [12].

The function of the axonally located mRNAs is still a matter of speculation. Recent results demonstrate that they are not subject to translation [9]. Yet, the axonal transcripts might be involved in the regulation of translation in the cell body. It has been proposed that mRNA localized to the axonal domain might represent a pool of stored transcripts that can be reactivated upon demand. For instance, during prolonged osmotic challenge the peptide hormone pools are depleted and the demand for AVP is substantially higher than the supply by precursor synthesis. Following the release of the stimulus, the stored mRNAs might be transported back into the perikaryon and serve again as templates for translation in order to effectively refill the depleted peptide hormone pools in the nerve terminals. During overhydration, in contrast, AVP synthesis is drastically diminished after 3 days and virtually shut off after 7 days of AVP V2 receptor agonist infusion [17]. As demonstrated here the axonal contents of the corresponding mRNA is reduced in parallel, presumably because the peptide hormone pools are sufficiently filled and any additional peptide hormone precursor synthesis with the aid of stored mRNAs is not required.

Acknowledgements: We thank Anke Peters (University of Hamburg) and Birthe Lynderup (University of Copenhagen) for expert technical assistance. This work was supported by the A.P. Møller, Alfred Benzon and Aage Louis-Hansen Foundation (to P.C.S. and N.A.T.) as well as the Deutsche Forschungsgemeinschaft (to E.M.) and the Human Frontier Science Program (to D.R.).

References

[1] Richter, D., Meyerhof, W., Morley, S.D., Mohr, E., Fehr, S. and Schmale H. (1988) in: The Roots of Modern Biochemistry (Kleinkauf, H., von Döhren, H. and Jaenicke, L., Eds.) pp. 305–321, De Gruyter, Berlin.
[2] Mohr, E., Morley, S.D. and Richter, D. (1990) in: Neuropeptides and their Receptors (Schwartz, T.W., Hilsted, L.M. and Rehfeld, J.F., Eds.) pp. 74–91, Alfred Benzon Symposium 29, Munksgaard, Copenhagen.
[3] Carter, D.A., Pardy, K. and Murphy, D. (1993) Cell. Mol. Neurobiol. 13, 87–95.
[4] Carrazana, E.J., Pasieka, K.B. and Majzoub, J.A. (1988) Mol. Cell Biol. 8, 2267–2274.
[5] Carter, D. and Murphy, D. (1989) J. Biol. Chem. 264, 6601–6603.
[6] Zingg, H.H., Lefebvre, D.L. and Almazan, G. (1988) J. Biol. Chem. 263, 11041–11043.
[7] Jirikowski, G.F., Sanna, P.P. and Bloom, F.E. (1990) Proc. Natl. Acad. Sci. USA 87, 7400–7404.
[8] Mohr, E., Zhou, A., Thorn, N.A. and Richter, D. (1990) FEBS Lett. 263, 332–336.
[9] Mohr, E., Morris, J.F. and Richter, D. (1995) Proc. Natl. Acad. Sci. USA 92, 4377–4381.
[10] McCabe, J.T., Lehmann, E., Chastrette, N., Hänze, J., Lang, R.E., Ganten, D. and Pfaff, D.W. (1990) Mol. Brain Res. 8, 325–329.
[11] Murphy, D., Levy, A., Lightman, S. and Carter, D. (1989) Proc. Natl. Acad. Sci. USA 86, 9002–9005.
[12] Mohr, E., Fehr, S. and Richter, D. (1991) EMBO J. 10, 2419–2424.
[13] Verbalis, J.G. and Drutarosky, M.D. (1988) Kidney Int. 34, 351–360.
[14] Svendsen, P. and Hau, J. (1990) Experimental Animals and Animal Experiments (in Danish), Odense University Press, Denmark.
[15] Heierhorst, J., Morley, S.D., Figueroa, J., Krentler, C., Lederis, K. and Richter, D. (1989) Proc. Natl. Acad. Sci. USA 86, 5242–5246.
[16] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Lab. Press, Plainview, New York.
[17] Robinson, A.G., Roberts, M.M., Evron, W.A., Verbalis, J.G. and Sherman, T.G. (1990) J. Clin. Invest. 86, 1023–1029.
[18] Chooi, K.F., Carter, D.A. and Murphy, D. (1992) Cell. Mol. Neurobiol. 12, 557–567.
[19] Jackson, R.J. and Standart, N. (1990) Cell 62, 15–24.
[20] Wickens, M. (1990) Trends Biochem. Sci. 15, 320–324.