International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Expression of Tick Receptor Protein in *Xenopus laevis* Oocyte

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Abstract: ***Mature Xenopus oocytes have diameters of 1 to 1.2mm. They are surrounded by a noncellular, fibrous vitelline layer and by several layers of follicular cells (Dumont et al., 1978) Fig. 1. Xenopus oocytes have been extensively used to study biogenesis, functional architecture, and modulation of plasma membrane expression of mRNAs encoding membrane receptors or ion channels. In 1971, Gurdon et al. reported that Xenopus oocytes translate foreign mRNA. Xenopus oocytes are capable of translation and post-translational modification (Ghysdael et al., 1977), phosphorylation (Gedamu et al., 1978) and glycosylation (Colman et al., 1981; Lund et al., 1986). A Xenopus oocyte can also assemble the subunits of a receptor and insert them into its surface membrane (Parker et al., 1985) Sumikawa et al. (1981) demonstrated the presence of binding sites for a-bungarotoxin when oocytes were injected with mRNA from the electric organ of Torpedo marmorata. Barnard et al. (1982) presented the first electrophysiological evidence for the expression of functional nAChR in Xenopus oocytes. Expressed protein can be detected as long as two weeks after RNA injection (Lerma et al., 1988). The Xenopus oocyte technique has been extended to examine the properties of other receptors . Gunderson et al. (1983,1984,1986); Miledi et al. (1982); Sumikawa (1984); Parker et al. (1984,1986) and, Sholfield et al. (1987) reported the successful expression of serotonin, dopamine, GABA and glycine receptors. Saito et al. (1987) reported for the first time that Xenopus oocytes successfully express glutamate receptors from lobster (Palinurus japonicus) abdominal muscle. L-glutamate responses in oocytes injected with lobster muscle RNA have been also reported by Kawai et al. (1989). Fraser et al. (1990) published that amino acid receptors expressed in Xenopus oocyte after " microinjection of mRNA from the leg of locust Schistocerca gregaria. Marshal et al. (1990) found the complete sequence of a locust (Schistocerca gregaria) nervous system nAChR subunit (alpha-L1). RNA derived from alpha-L1 cDNA when injected alone in Xenopus oocyte resulted in the expression of functional nAChR. Arena et al. (1991) recently reported that C. elegans poly A+ RNA injected into Xenopus oocytes expresses L-glutamate-sensitive chloride channels.***

Keywords: mRNA: messenger RNA, nACR: nicotinic acetylcholine receptor, DEPC: Diethyl pyrocarbonate, GABA: gamma aminobutric acid

1. Introduction

In recent years *Xenopus* oocyte has taken over a new role as a test tube for the study of the biogenesis, functional architecture and modulation of plasma membrane protein. Attempts were made to express mRNA from tissue of tick embryo and locust leg muscle in to *Xenopus* oocyte for pharmacological studies. The objective of this study was to develop a system that would allow a study of the pharmacology of arthropod transmitter receptor proteins. In the whole series of experiments, 40 batches of oocytes were injected and tested for receptor protein expression. Xenopus oocytes failed to translate RNA faithfully and efficiently from these sources. Rat brain RNA injected oocytes used as control, expressed routinely.

2. Material and Method

RNA extraction and purification and microinjection:
Total RNA was extracted from locust leg; rat brain and from 1,3,5,7,9,11, 13,15,17, and 19-day eggs embryo of *Boophilus microplus.* The method used was described by Chomczynski and Sacchi (1987) (Fig. 2). *Boophilus* eggs were obtained from Schering Agrochemical Chesterford park Cambridge. The eggs were rapidly frozen in liquid nitrogen and stored at -80^0 C. At the time of extraction the eggs were weighed and l gm of eggs were homogenised in 4M guanidium thiocyanate. This solution is an RNAase inhibitor. A phenol- chloroform extraction method was used to separate RNA from protein using reagents I -IV described above. A second precipitation step with isopropanol was undertaken and then the RNA was resuspended in 75% ethanol / 0.3M sodium acetate solution. There then followed a third precipitation step. The resultant pellet was washed in 95 % ethanol and then desiccated before being re-suspended in autoclaved, DEPC- treated, sterile water. mRNA was purified by using Dynabead oligo- dt (deoxythymidine) column. The quality of RNA and mRNA was determined, (I) by checking optical density using aspectrophotometer, (II) using a 1% agarose gel electrophoresis, and (III) by in vitro translation in rabbit reticulocyte lysate.

Female Xenopus laevis (Fig 3 A and 3 B) were obtained from Blades Biological, Kent, and maintained in 20-gallon glass tanks of circulating, clean, chlorine-free water. The tanks were divided by perspex sheets to keep the animals separate, so that individuals could be identified. This was necessary because the quality of oocytes differed from animal to animal. The animals were exposed to a 12- hour light/dark cycle at 20^0 C – 22 ^{0} C and fed on small pieces of fresh pieces of heart, mealworms, maggots and locust hoppers.Attempts have been made to express mRNA from arthropod excitable tissue in Xenopus oocyte in order to study the pharmacology of arthropod transmitter receptor proteins.

In the whole series of experiments, 40 batches of oocytes were micro injected by using a pateform (FIG. 4) and tested for receptor protein expression. Unless otherwise stated, -150 oocytes were injected in each batch with total RNA or

mRNA of tick and -10 oocytes each with total RNA of locust leg and of rat brain. Oocytes were treated with Con A (10-6M) for 30 min prior to test for receptor protein expression. L-glutamate, L-quisqualate, N-methyl-D-aspartate and DL-ibotenate were bath-applied to oocytes after 2 to 21 days post-injection with RNA.

Electrophysiology: The microinjected oocytes were transferred to a perfusion bath containing experimental saline flowing at 3m1/min (Fig.5) Two small pins were placed in the bath to hold the oocytes in position. The expression of neurotransmitter receptors was examined from 1 - 21 days post-injection using a two-electrode voltage clamp (Axoclamp 2A, Axon Instruments) Electrodes (GC 15OTF-10, Clark Electromedical Instruments) were pulled to give a resistance between 0.8 MS2 -1 MSZ and were filled with 3M KC1.

3. Result

The objective of this part of project was to develop a system that would allow a study of the pharmacology of arthropod transmitter receptor proteins. In the whole series of experiments, 40 batches of oocytes were injected and tested for receptor protein expression. Unless otherwise stated, -150 oocytes were injected in each batch with total RNA or mRNA of tick and -10 oocytes each with total RNA of locust leg and of rat brain. Oocytes were treated with Con A (10-6M) for 30 min prior to test for receptor protein expression. L-glutamate, L-quisqualate, N-methyl-D-aspartate and DL-ibotenate were bath-applied to oocytes after 2 to 21 days post-injection with RNA. Oocytes injected with distilled water failed to exhibit any response when tested with L-glutamate. However, oocytes injected with total RNA (50n1/oocyte) from rat showed expression (96% success rate) giving inward currents of 120nA - 220nA at a membrane potential of -60mV (Fig. 6). Oocytes injected with tick or locust RNA exhibited a very poor rate of expression (<0.2% and <0.8% respectively; Table: 1). In attempt to improve the expression rate, oocytes were de-folliculated and microinjected (50n1/oocytes) with RNA 3 times at 2-day intervals. Oocytes injected with tick embryo message failed to respond between 2-14 days post-injection. However, 7 oocytes 16 days after post-injection with total RNA froml 8 day old embryos of tick exhibited small responses (' . l OnA) to L-glutamate or L-quisqualate (Fig. 7) In another set of experiments, oocytes were co-injected with RNA obtained from 1,3,4,7,9,11,13,15,17,19 days-old tick embryos but no improvement in expression was achieved. To exclude the possibility that antibiotics might be inhibiting synthesis of arthropod proteins, oocytes were maintained in Barth saline containing no antibiotics. The saline was changed twice every day under aseptic conditions and oocytes were successfully maintained for three weeks after microinjection. When tested electrophysiologically, the oocytes had resting potential of -50mV to - 60mV. None of the oocytes (n=200) responded when tested with L-glutamate, N-methyl-D-aspartate, L-quisqualate or DL-ibotenate. The genome of Xenopus contains two heat shock gene hsp 30, and hsp 70. However, synthesis of hsp 70 protein is not detected unless the temperature at which the oocyte is incubated is shifted from 18°C to 370C (Bienz et al., 1982). Although the function of Xenopus heat shock protein is not known, an interesting function has been ascribed to mammalian hsp 73 protein. This protein apparently has the ability to bind the poly(A) tail of mRNA and as consequence could regulate the stability or translatability, or both, of mRNA that have 3' poly(A) tails (Schonfelder et al., 1985). Oocytes were injected with total RNA and mRNA from different ages of tick embryos and maintained at 180C for 24h. The oocytes were then transferred to an incubator at 370C for 2-5h. The oocytes were subsequently tested with a range of glutamate agonists, but none exhibited responses. Thus, it appears that failure to obtain good expression of tick embryos or locust muscle.

4. Discussion

The *Xenopus* oocyte expression system is a versatile and informative method for studying the pharmacological properties of EAARs. This model system faithfully translates foreign RNA from vertebrate sources, however it failed to translate invertebrate mRNA efficiently. The reason for this difference remains unclear.

Attempts to increase the level of receptor expression by doubling the concentration of RNA (2µg/µ1), by injecting three times in one week, by extraction of RNA from different developmental stages of embryos of tick, by removing antibiotics from media and by heat shock to initiate protein synthesis were unsuccessful. The pharmacological properties of expressed receptor remained unclear due to very poor expression rate and small currents. This study suggests that *Xenopus* oocyte is a good model to study the pharmacology of vertebrate receptors but failed to translate invertebrate mRNA very efficiently. The reason for this failure of functional expression of protein of interest may be due to low abundance of the specific mRNA(s) in the source tissue or stability of the mRNA into the oocyte cytoplasm after injection. Some polypeptides may be improperly modified, folded or assembled, and therefore not reach the plasma membrane.

An in vitro (rabbit reticulocyte lysate) translation system was used to compare the RNA from rat brain with the invertebrate RNAs. The result of this study suggest that the capacity of translation of vertebrate and invertebrate message was almost the same. Thus, it appears that failure to obtain good expression of RNA of tick embryos RNA may be due to some unknown failure of Xenopus oocyte to translate arthropod mRNA and further study is clearly needed.

References

[1] Barnard, E. A; Miledi, R. and Sumikawa, K. (1982) Translation of exogenous mRNA coding for nAchR in Xenopus oocytes. Proc. R. Soc. (Lond) B. 215: 241.
[2] P Chomczynski et al. Anal Biochem (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction
[3] Colman, A; Lane, C. D; Craig, R; Boulton, A; Mohun, T and Morser, J. (1981) The influence of Topology and Glycosylation on the fate of heterologous secretory proteins made in Xenopus oocytes. Eur. J. Biochem. 113: 339-348.

[4] Fraser, S. P; Djamgoz, M. B. A; Usherwood, P; O'Brien, J; Darlison, M. G. and Barnard, E. A. (1990) Amino acid receptors from insect muscle: electrophysiological characterization in Xenopus oocytes following expression by injection of mRNA. Mol. Brain.. Res. 8: 331-341

[5] Ghysdeal, J; Hubent, E; Travnicek, M; Bolognesi, D. P; Burney, A; Cleuter, Y; Huez, G; Kettman, R; Marbaix, G; Portelle, D; and Chantrenn, H. (1977) Frog oocytes synthesize and completely process the precursor polypeptide to virion structural proteins after microinjection of avian myeloblastosis virus RNA. Proc. Natl. Acad. Sci. U. S. A. 74: 3230-3253.

[6] Gedamu, L; Dixon, G. H. and Gurdon, J. B. (1978) Studies of the injection of Poly (A)+ protamine mRNA into Xenopus laevis oocytes. Exp. Cell Res. 117: 325-334.

[7] Gunderson, C. B; Miledi, R. and Parker, I. (1983) Serotonin receptors induced by exogenous mRNA in Xenopus oocytes Proc. R. Soc. Lond. B. Biol. Sci. B 219: 103-109.

[8] Gunderson, C. B; Miledi, R. and Parker, I. (1984a) Messenger RNA from human brain induces drug and voltage-operated channels in Xenopus oocytes. Nature (London) 308: 421-424.

[9] Gunderson, C. B; Miledi, R. and Parker, I. (1984b) Glutamate and kainate receptors induced by rat brain mRNA in Xenopus oocytes. Proc. R. Soc. Lond. B. Biol. Sci.. 221: 127-143.

[10] Gunderson, C. B; Miledi, R. and Parker, I. (1986) Voltage- dependence of human brain glycine receptor-channels in Xenopus oocytes. J. Physiol; Lond. 377: 40p.

[11] Kawai, N; Saito, M. and Ohsako, S. (1989) Differential expression of glutammate receptors in Xenopus oocytes injected with mRNA from lobster muscle. Neurosci. Lett. 93: 203-207.

[12] Lerma, J; Kushner, L; Bennet, M. V. L. and Zukin, S (1988) NMDA. kainate and quisqualate receptors of rat brain expressed in Xenopus oocytes: summation experiments indicate that each gates separate channels. Neurosci. Abstr. 14: 1194.

[13] Marshall, J; Buckingham, S. D; Shingai, R; Lut, G. G; Gossey, M. W; Darlison, M. G; Sattelle, D. B' and Barnard, E. A. (1990) Sequence and functional expression of a single alpha-subunit of an insect nicotinic acetylcholine receptor. EMBO. J. 9: 4391-8.

[14] Miledi, R; Parker, I. and Sumikawa, K. (1982) Synthesis of cat muscle acetylcholine receptors by Xenopus oocytes, Biomed. Res. 3: 390-399.

[15] Miledi, R; Parker, I. and Sumikawa, K. (1982) Synthesis of chick brain GABA receptors by frog oocytes. Proc. R. Soc. Lond. B 216: 509-516.

[16] Parker, I; Gunderson, C. B and Miledi, R (1984) A transient inward current ellicited by hyperpolarization during serotonin activation in Xenopus oocyte. Proc. R. Soc. Lond. B 223: 279- 292.

[17] Parker, I; Gunderson, C. B and Miledi, R (1984) A transient inward current ellicited by hyperpolarization during serotonin activation in Xenopus oocyte. Proc. R. Soc. Lond. B 223: 279- 292.

[18] Saito, M; Ohsako, S; Deguchi, T. and Kawai, N. (1987) Glutamate receptors expressed in Xenopus oocytes by mRNA from invertebrate muscle. Mol. Brain. Res. 3: 83-87.

[19] Sumikawa, K; Houghton, M; Emtage, J. S; Richards, B. M. and Barnard, E. A. (1981) Xenopus oocytes injected with mRNA for a human ß-cell line: active multi subunit AchR assembled by translation of heterologous mRNA in Xenopus oocytes. Nature (Lond.). 292: 862.

[20] Sumikawa, K; Parker, I. and Miledi, R. (1984) mRNA from rat brain induced noradrenaline and dopamine receptors in Xenopus oocytes. Proc. R. Soc. (Lond). B223: 255-266