^{35}Sulfur Incorporation in Neural Membrane Protein Involved in *Periplaneta americana* Chemoreception

Classical sulfhydryl reagents have been repeatedly shown to alter the energy-transfer interactions between feeding-inhibitory messengers and the nerve-membrane receptor protein in *Periplaneta americana*[1–6]. The receptor protein was originally isolated by FERKOVICH and NORRIS[7], and was further characterized electrophoretically by SINGER et al.[6,8,9] and polarographically by ROZENTAL and NORRIS[10,11]. Because of the predictable disruption of the receptor function of this protein by sulfhydryl reagents[3,5,6,10,11] ^{35}S incorporation into the receptor protein was studied in vivo using (^{35}S) cystine and the results are presented in this paper.

Materials and methods. (^{35}S) L-cystine dihydrochloride solution (Amersham/Searle), diluted with 0.9% NaCl solution to yield about 260,000 cpm/µl (Packard Tri-Carb, Model 3380), was injected into the abdominal haemocoel of individual *P. americana* males. Groups of 5 injected insects per treatment were held for 1 h to 35 days before protein extraction. Each cockroach of a group had its head severed at the neck, rinsed in 0.9% saline at 0 °C, transferred to 34 ml of saline at 0 °C, macerated in a Teflon-glass homogenizer for 15 min, filtered through glass wool with mild suction and centrifuged (max. 100,000 × *g*) for 30 min. This entire process yielded 33 ml of a supernatant, S_1, and a pellet. The pellet was rehomogenized in 33 ml of saline at 0 °C and centrifuged to yield supernatant S_2, and a pellet. The second pellet was further homogenized in 3.5 ml of 0.6% Triton X-100 in 0.9% saline to yield a supernatant, T_1, and the final pellet. All solutions were made with double glass distilled, N_2 saturated water. Replicated measured aliquots of each supernatant were counted directly in a 10 ml cocktail (500 ml toluene, 500 ml methyl cellusolve, 0.3 g dimethyl POPOP and 5.5 g PPO). Quenching was 56% as compared to the normalization standard. The final pellet was digested with 0.2 ml of 30% H_2O_2 overnight at 62 °C before counting.

Replicates (0.3 ml) of S_1 and T_1 solubilized proteins were electrophoreted in pH 7.1 systems, fixed/stained with 0.5% Amido Black in 7% acetic acid and destained in 7% acetic acid prior to slicing of the gels for counting the radioisotope label[6,9].

Results and discussion. The major ^{35}S peak (solid black squares, Figure 1) among the electrophoreted T_1 protein bands matched the major ^{14}C peak previously found when T_1 proteins were exposed in vitro to 2-methyl (^{14}C)-1,4-naphthoquinone, a feeding inhibitor for *P. americana*[6]. The same protein band also contained the ^{14}C peak from the messenger quinone when T_1 was prepared, electrophoreted and counted after in vivo exposure of cockroach antennae to the ^{14}C quinone messenger[7,9]. Thus, current experimental results confirm the electrophoretic characteristics of the chemoreceptor protein and demonstrate selective ^{35}S incorporation into this membrane-bound receptor following injection of ^{35}S-cystine into the abdomen. Incorporation of ^{35}S label in this receptor band occurred within 1 h (Figure 1, solid black squares). It peaked in the total Triton extract (Figure 1, solid black circles) at about 24 h, remained virtually unchanged for 9 days, and had declined significantly by 23 days. However, the pellet-bound ^{35}S cpm showed a progressive increase through 35 days after injection (Figure 2).

The incorporation of ^{35}S into the S_1 extractables is shown in Figure 3 (solid black circles). ^{35}S-Protein incorporation was best documented by slicing gels and counting slices for ^{35}S after electrophoresis and protein fixation and staining, because considerable non-protein ^{35}S was present in the crude saline extract. Proteins, electrophoretically distinct from the receptor, in the saline fraction (Figure 3, open circles and squares), showed ^{35}S incorporation during 1–6 h which closely paralleled that in the receptor (Figure 1, solid black squares). Some of these soluble proteins may be precursors (e.g., subunits) for the membrane-associated chemoreceptor protein.

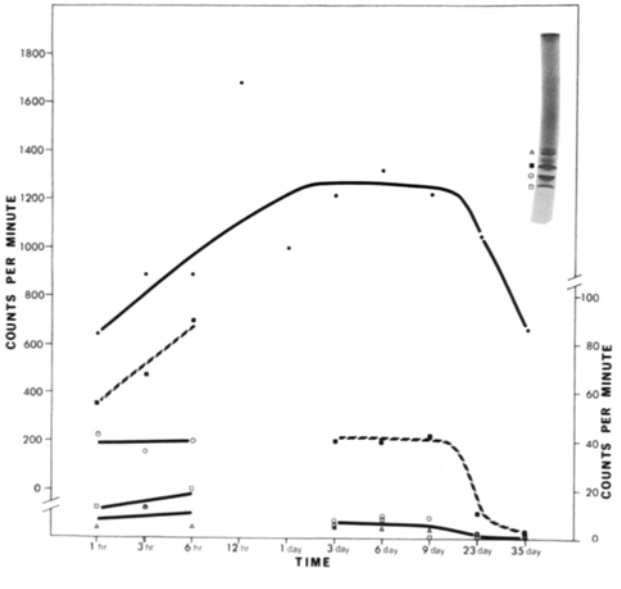

Fig. 1. ^{35}S recovery over time from total Triton-solubilized extract from head macerate of 5 *P. americana* after abdominal injection of 124,231 cpm of ^{35}S cystine per insect (solid black circles, cpm scale on left margin). Recovered ^{35}S from each protein band is after electrophoreting 0.3 ml of the total Triton extract, and fixing and staining gel before slicing and counting. Symbol for each electrophoreted band is shown beside a photographed gel. Counts per band at 1–6 h represent the heads of 5 insects, each initially injected with 520,000 cpm (cpm scale on the right margin). Counts per band at 3–35 days represent the heads of 5 insects, each initially injected with 124, 231 cpm (cpm scale is on the right margin). Cpm's are corrected for the half-life of ^{35}S.

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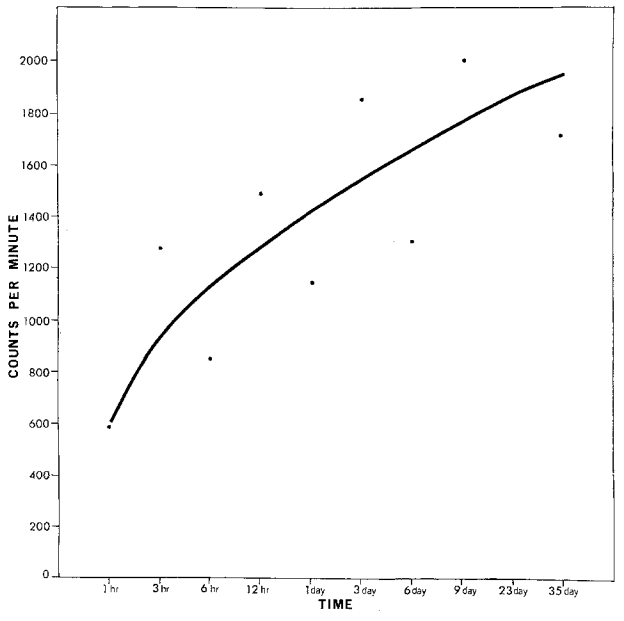

Fig. 2. ^{35}S recovery over time from the total residual pellet from heads of 5 insects, each insect initially injected with 124,231 cpm.

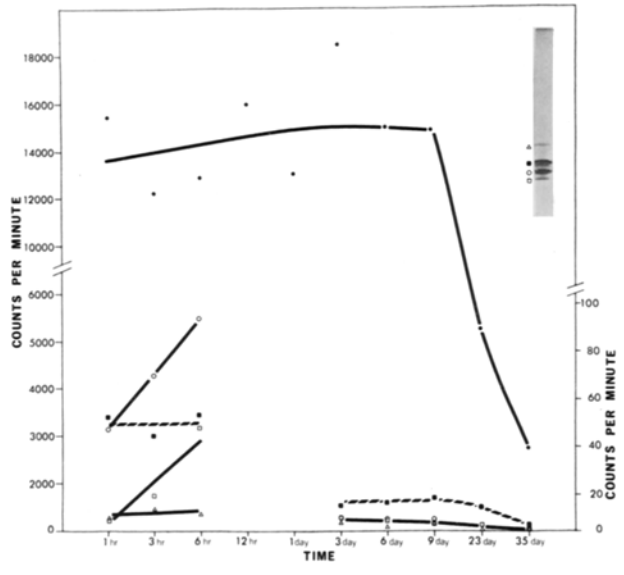

Fig. 3. Same as Figure 1 except involves the total saline-soluble fraction, and electrophoreted protein bands.

After 3 days, the greatest ^{35}S incorporation into soluble protein was in the band (Figure 3, solid black squares) which shows the electrophoretic characteristics of the membrane-associated chemoreceptor protein in the T_1 extract (Figure 1, solid black square).

Although ^{35}S incorporation into these protein fractions has been demonstrated, a fraction of the intitial rapid label uptake (1 to 24 h) may have been due to a formation of mixed disulfides. Reduction with excess thiol, column fractionation and electrophoresis of these ^{35}S incorporated extracts[12] demonstrated that the label recovered in the T_1 extract after 3 days of in vivo incubation was essentially all incorporated into the polypeptide chain of the receptor protein.

ROZENTAL and NORRIS[10,11] have shown that messenger quinones cause characteristic conformational changes in the T_1 receptor protein, but not in S_1 proteins. The T_1 receptor protein is an integral component of the nerve membrane[7,9] and its subunits become characteristically aggregated or unfolded in response to specific messengers[10,11]. Though characteristic aggregation or unfolding in response to messengers has not been found with S_1 proteins[10,11], the S_1 proteins will bind messenger quinones under physiological conditions[12]. Our tentative interpretation is that the ^{35}S labeled S_1 proteins which are apparent precursors (subunits) of the membrane-associated receptor contain thiol groups and bind messenger, but that these saline-soluble proteins lack the abilities of the membrane-bound T_1 receptor to predictably change conformation in response to each messenger, as occurs in the energy-transferring mechanism in the sensory nerve membrane during chemoreception.

Resumen. (^{35}S) cistina injectada en la cavidad sanguínea abdominal fue incorporada en una proteína quemoreceptora aislada de tejido nervioso. La incorporación empezó durante la primera hora, llegó a una máximo a las 24 h, se mantuvo a este nivel por 9 días y a los 23 días había declinado.

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[12] G. SINGER and D. M. NORRIS, unpublished data.

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Swelling of Brain Slices: Non-Osmotic Reversion Caused by Metabolic Intermediates

During in vitro incubation mammalian brain slices take up water[1]. The swelling increases in hypotonic solutions, in anoxia[2], after addition of inhibitors or uncouplers of the glucose metabolism[3], and finally in the presence of glutamate if the medium contains potassium[4]. A complete prevention of the swelling is not possible; but the water uptake is reduced, if the preparation of the slices is made under mild conditions[5], if efficient oxygenation is provided for[2], if chloride in the medium is substituted by isethionate[6], or if hypertonic solutions are used[7,8]. OKAMOTO and QUASTEL[9] observed almost

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