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BIOCHEMICAL STUDIES ON THE INITIATION OF ODOR SENSING
(U) MISSISSIPPI STATE UNIV MISSISSIPPI STATE R B KOCH
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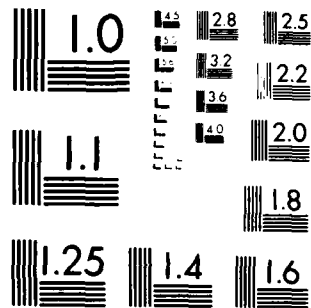
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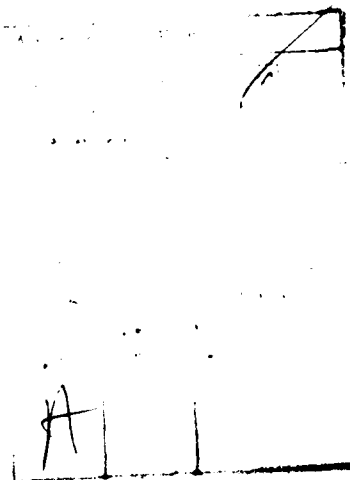
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ABSTRACT (cont.)

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Final Report

on

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List of Publications

Studies on Olfactory Tissue $\text{Na}^+ - \text{K}^+$ ATPase Activities.
Master of Science Thesis by Hernan Rossi, Dissertation
Abts. 1981. Advisor: R.B. Koch.

Effect of antibody to anisole binding protein on odorant
perturbation of $\text{Na}^+ - \text{K}^+$ ATPase activity.
R. B. Koch, Hernan Rossi and Steven Price
Chem. Senses 6, 95-99 (1981).

Odorant responses of $\text{Na}^+ - \text{K}^+$ ATPase activity by preparations
from paired turbinals of rat olfactory tissue.
Hernan Rossi and Robert B. Koch.
Chem. Senses 6, 113-117 (1981).

Odorous chemical perturbation of $\text{Na}^+ - \text{K}^+$ dependent ATPase
activities. Effects on native and lipid-substituted
preparations from individual turbinals from dog
olfactory tissue.
Thomas D. Dreesen and R. B. Koch
Biochem. J. 203, 69-75 (1982).

In Vitro response of ATPase activities in tissue subcellular
particle preparations to a series of mono-unsaturated
 C_{18} fatty acids.
Robert B. Koch
Biochem. Pharm. 31, 867-872 (1982).

Effect of chain length and degree of unsaturation of naturally
occurring fatty acids on $\text{Na}^+ - \text{K}^+$ ATPase Activity.
Robert B. Koch and Jeanne W. Smith.
Federation Proceedings 42, 1935 (1983) Abstract #1038.

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The overall purpose of this project was to investigate the basic biochemical events which are responsible for the initiation of the process of odor sensing, in an attempt to understand our ability to sense and identify an infinite number of odorous compounds. It has been observed that even newly synthesized organic compounds (with sufficient volatility at room temperature) can be detected by the sense of smell. This fact has led the principal investigator to propose that specific receptors for odorous chemicals cannot be pre-existent in the olfactory epithelial tissue, and that odor sensing and identification is due to pattern recognition of multiple perturbations of nerve action potentials.

Changes in $\text{Na}^+ - \text{K}^+$ ATPase activity from olfactory tissue nerve ending particle (NEP) preparations, but not from brain preparations, in response to odorants (Koch, 1971/72) gave the first indication that activity of this enzyme was, in some way, involved in the odor sensing process. Since that time, and especially during the period funded by this contract, research results have given further support for this hypothesis.

The specific aims of the original proposal were the following:

- Aim 1 - To determine patterns of changes in ATPase activities from (dog) olfactory NEP preparations to a set of odorants.
- Aim 2 - To determine the effects of detergent treatment and phospholipid replacement of olfactory tissue NEP preparations on odorant perturbations of the $\text{Na}^+ - \text{K}^+$ ATPase activity and its response to odorants.

Aim 3 - To isolate and purify $\text{Na}^+ - \text{K}^+$ ATPase from olfactory tissue NEP preparations.

Aim 4 - To determine the nature of the molecular structure of the purified enzyme (subunit composition, glyco-protein content, lipid requirements for activity, and requirements for odorant perturbations).

Aim 5 - If subunits can be manipulated without loss of enzyme activity, to begin an investigation of the effects of subunit interchange between preparations from olfactory sensing tissues which show differences in response to selected odorants.

THE MOST SIGNIFICANT RESULTS OBTAINED IN STUDIES
PERFORMED TO ACHIEVE THESE AIMS ARE SUMMARIZED BELOW:

Summary of Results

1. As reported by Koch, Rossi, and Price (1981), antibody to dog olfactory epithelium was capable of specifically preventing stimulation of olfactory tissue $\text{Na}^+ - \text{K}^+$ ATPase activity by an odorous chemical. (Aim 1)
2. The response of $\text{Na}^+ - \text{K}^+$ ATPase activity of NEP preparations from left-right paired turbinals in the olfactory capsule showed a lack of bilateral symmetry of response to odorants (Rossi and Koch, 1981; Dreesen and Koch, 1982). (Aim 1)

3. Na^+-K^+ ATPase activity of NEP preparations from individual turbinals of one animal responded quite differently to a single odorant. Each different odorant tested produced a different and apparently unique enzyme response pattern. (Aim 1)
4. Partial delipidation of NEP preparations and replacement with a single type of phospholipid caused the odorant response patterns of Na^+-K^+ ATPase activity to change (Dreesen and Koch, 1982). Individual turbinal NEP preparations, when substituted with the same phospholipid, showed similar Na^+-K^+ ATPase response to the same concentration of a particular odorant even though the same preparations before phospholipid exchange had very different enzyme activities (Aim 2). These results suggest that the type of phospholipid in the Na^+-K^+ ATPase lipoprotein complex could be an important factor in determining odorant interaction with the complex, and in enzyme activity response patterns.

More recent studies have focused on purification of Na^+-K^+ ATPase using the detergent sodium dodecylsulfate (SDS) (Aim 3). A brief description of the modification of the Jørgensen (1974) procedure as presently employed is given below.

A cow olfactory NEP preparation was centrifuged at 13,000xg for 20 min and the resulting pellet resuspended in buffer (50mM imidazole pH 7.5, 2 mM EDTA, 3 mM ATP) to give a protein concentration of approximately 1.5 mg/ml. After homogenization, 1.5 ml aliquots were incubated for 45 min at

room temperature with varying concentrations of SDS (0.14 to 0.56 mg/ml final concentration). Solutions were stirred after incubation and 1.0 ml samples were layered on sucrose gradients consisting of 2.0 ml 24.9% sucrose and 1.3 ml 15% sucrose. The samples were centrifuged for 113 min at 49,000 rpm in an L5 Beckman Ultracentrifuge using a SW 50.1 rotor. Dense bands which formed at the sucrose interface were removed with long barrel micropipets. These fractions were diluted with 0.32M sucrose solution and pelleted by centrifugation. Pellets were resuspended and homogenized in 0.6 ml buffer solution (25 mM imidazole pH 7.5, 1 mM EDTA). Proteins were determined by the method of Lowry et al. (1951). This general procedure for SDS solubilization of cow olfactory tissue NEP preparations differed from that of Jørgensen (1974) in the following ways: (1) Jørgensen used large quantities (80 ml) of kidney microsomes for SDS treatment; however, only 1.5 ml aliquots of cow olfactory tissue NEP preparations were used because of limited size of tissue fractions. (2) Jørgensen found that optimum purification conditions for his kidney microsomal preparations were 1.4 mg/ml protein treated with 0.56 mg/ml SDS; however, cow olfactory NEP $\text{Na}^+ - \text{K}^+$ ATPase activity was inactive under these conditions. SDS (0.14 to 0.32 mg/ml) has been found to be more effective in purification of cow olfactory tissue NEP fractions at similar protein content. (3) Greatest $\text{Na}^+ - \text{K}^+$ ATPase specific activity of the SDS-treated cow olfactory tissue preparation was found at the interfacial

band after density gradient centrifugation, while Jørgensen found highest $\text{Na}^+ - \text{K}^+$ ATPase specific activity in the pellet from the kidney microsomal SDS-treated sample. Jørgensen (1974) reported specific activities of 1,000 to 2,000 for kidney SDS sediment preparations. In our preliminary investigations using the modified Jørgensen procedure, fractions of SDS-treated olfactory tissue preparations that were recovered as bands at the sucrose interface after density gradient centrifugation yielded activities of 30-35 $\mu\text{mol Pi mg}^{-1} \text{ protein hr}^{-1}$; much less than brain or kidney preparations. However, it should be noted that Jørgensen used a kidney preparation with an initial specific activity of over 200. However, the olfactory tissue starting fractions had specific activities between 1 and 2 $\mu\text{mol Pi mg}^{-1} \text{ protein hr}^{-1}$. It is possible that the turnover number of the $\text{Na}^+ - \text{K}^+$ ATPase from olfactory tissue is much lower than from brain and kidney preparations. In a recent study by Sen and Pfeiffer (1982), partial purification of pig lens $\text{Na}^+ - \text{K}^+$ ATPase resulted in specific activities of only 0.3 to 0.5 $\mu\text{mol Pi mg}^{-1} \text{ protein hr}^{-1}$ which was a 100-fold increase over the specific activity of the homogenate.

Slab gel SDS-electrophoretic analysis (Laemmli, 1970) of the small samples of SDS treated olfactory tissue preparations showed a number of bands (as would be expected from a partially purified fraction) but distinct bands at $\text{Mr} \sim 100,000$ and $\sim 50,000$ which have been reported for purified $\text{Na}^+ - \text{K}^+$ ATPase from other tissue sources.

A modified version of the Jørgensen procedure was reported by Mayrand et al (1982) for the purification of rat brain Na^+-K^+ ATPase. Although it is described as a simpler procedure than that of Jørgensen (1974) because density gradient centrifugation is omitted, four separate centrifugations are required. In a preliminary study using a cow brain NEP fraction, purification of the Na^+-K^+ ATPase by this method (Mayrand et al., 1982) resulted in only a 2-fold increase in specific activity. Therefore, the Jørgensen procedure has been selected for further study. (With the latter method, a 10-fold increase in specific activity for brain and a 23-fold increase for cow olfactory tissue Na^+-K^+ ATPases have been obtained as noted earlier.)

Other recent studies have compared responses to odorants by Na^+-K^+ ATPase activities from cow olfactory and insect antennal preparations (Table I). It was found that cow olfactory Na^+-K^+ ATPase activity responded quite differently to odorant (insect pheromone components) than the insect enzyme (Table I). There was apparent agreement in sensitivity response with behavioral, electrophysiological and biochemical (ATPase) studies on insects (Linn and Gaston, 1981; this report Table I). Na^+-K^+ ATPase activities of male and female insect (T. ni moths) antennal preparations were strongly inhibited at high ($1 \times 10^{-4}\text{M}$) pheromone concentrations (Table II). However, stimulation of enzyme activity responses by lower concentrations of odorant ($1 \times 10^{-9}\text{M}$ or 10^{-12} mole per ml) was only observed with the NEP fraction of the male moth antennae (Table II).

In another recent study using cow olfactory tissue, NEP preparations from three sections of a single turbinal were obtained. $\text{Na}^+ - \text{K}^+$ ATPase activity responses to odorants were found to be different for each section. Differences in patterns of response to odorants of the olfactory epithelium have also been measured electrophysiologically (MacKay-Sim, et al, 1982). Results from biochemical and electrophysiological studies indicate that unique patterns of response to odorants may be responsible for our ability to distinguish various odorous compounds.

The effects of various fatty acids (FA) on ATPase activities from brain NEP preparations were also investigated (1) because these are long hydrocarbon chained molecules similar to some of the odorous chemicals used in the olfaction experiments, and (2) because FA are components of the phospholipids associated with the $\text{Na}^+ - \text{K}^+$ ATPase in the membrane structure. Changes in $\text{Na}^+ - \text{K}^+$ ATPase activity as a result of the presence of a particular FA in the reaction mixture may indicate a disruption of the normal phospholipid-enzyme complex by the added FA. Very briefly, results of this study showed that enzyme inhibition by saturated FA increased as chain length decreased from 18 to 14 carbons. Conversely, enzyme inhibition by mono-unsaturated FA increased as carbon chain length increased from C_{14} to C_{18} . In all but one case (C_{14} and $\text{C}_{14:1}$), the mono-unsaturated FA's were more inhibitory than the corresponding saturated FA's. Increasing the number of double bonds from 1 to 3 for C_{18} FA's did not cause increased inhibition of the enzyme activity.

Methylation of oleic acid ($C_{18:1}$), to remove the negative charge of the $-COO^-$ group, resulted in complete loss of the enzyme inhibition observed with the free fatty acid, suggesting the necessity of a charge for enzyme inhibition. The importance of a charge was also investigated in a recent study using various phospholipids, cholestanyl derivatives, and dioleoyl phosphatidic acid on Na^+-K^+ ATPase activities from a mouse brain NEP preparation. Preliminary results indicate that neither the cholestanyl derivatives (no charge) nor the various phosphatidylcholines (positive charge) had any significant effect on the enzyme activity. However, dioleoyl phosphatidic acid, (negative charge) caused significant inhibition of the Na^+-K^+ ATPase activity.

It is proposed that disruption of the secondary bonding forces between the phospholipid-enzyme complex results in the inhibition of enzyme activity. As stated previously, the intact complex has been shown to be important for active Na^+-K^+ ATPase (Simpkins and Hokin, 1973; Hilden and coworkers, 1974-1976; Walker and Wheeler, 1975; and Kimelburg and Papahadjopoulos, 1974), and in olfactory tissue, for stimulation by odorants to occur (Dreesen and Koch, 1982), perhaps because it maintains proper conformation of the enzyme for activity as suggested by simpkins and Hokin, 1973).

It is proposed that stimulation of Na^+-K^+ ATPase from olfactory tissue NEP preparations may occur by odorant interaction with the lipoprotein complex of the small subunit

(which may act as a Na^+ ionophore). The odorant perturbation of the ionophore subunit "turns on" or activates the large subunit which is the Na^+ pump. Thus it is proposed that Na^+ pumping is actually turned on by the odorant perturbation of a small subunit - not by the depolarization of the membrane. The Na^+ pump continues to act until proper membrane potential is re-established. This conclusion was necessarily reached because in tests on NEP preparations, no potential gradient can be established in the "leaky" vesicles and Na^+-K^+ ATPase activity in our assay remains constant for 10-30 minutes.

Because odorants elicit different degrees of stimulation of Na^+-K^+ ATPase activity, even from sections within one turbinal, it is proposed that odor sensing is based on pattern recognition. Each odorant produces a specific pattern of enzyme perturbations across the epithelium of the turbinals which leads to depolarization of certain olfactory bipolar nerve cells. The nerve impulses are processed in the olfactory bulb and then transmitted to the area of the brain which interprets the unique pattern as a specific odor.

The above proposed mechanisms resulting from studies supported by this contract will require further research efforts to confirm and extend our understanding of molecular interactions between odorants and purified lipoprotein complexes of olfactory tissue Na^+-K^+ ATPase preparations. This proposed mechanism is unique. It is hoped that these unique findings will stimulate others to conduct similar research on

the mechanism of odor sensing, as progress by the Principal Investigator will be affected by lack of renewal of this research project. It is further proposed that the understanding of the molecular specificity of the $\text{Na}^+ - \text{K}^+$ ATPase response to odorants will lead to a new and unique form of sensing that will have broad application and sensitivity to very low concentrations of chemical odorants.

TABLE I
PERCENT CHANGE IN Na^+/K^+ ATPASE ACTIVITY BY I. NI. PHEROMONE COMPONENTS ON
B FRACTION SUBCELLULAR PARTICLE PREPARATIONS FROM VARIOUS SOURCES.

MOLAR CONC.	MALE CABBAGE LOOPER ANTENNAE		COW OLFACTORY TISSUE		MOUSE BRAIN TISSUE	
	Z7:12AC	12AC	Z7:12Ac	12AC	Z7:12AC	12AC
1×10^{-4}	-51%	-59%	+32% ± 2 n=4	+45% ± 4 n=6	-32% ± 2 n=3	+1% $\pm .5$ n=5
5×10^{-5}	-48%	---	---	---	---	---
1×10^{-5}	+8%	+7%	+18% ± 1 n=4	+32% ± 3 n=4	-31% ± 1 n=3	+2% $\pm .5$ n=5
5×10^{-6}	+19%	---	---	---	---	---
1×10^{-6}	+4%	+13%	+7% ± 3 n=5	+9% ± 2 n=3	-2% ± 1 n=3	+1% $\pm .5$ n=4
1×10^{-7}	+3%	-1%	+3% ± 1 n=5	+6% ± 1 n=3	---	+1% ± 1 n=4
1×10^{-8}	+7%	---	---	---	---	---
1×10^{-9}	+24% ± 1 n=2	+9%	+1% ± 3 n=2	+5% ± 1 n=3	-3% ± 2 n=2	-1% ± 1 n=3
CONTROL SP. ACT.=	7.4 $\pm .3$ n=4	7.4 $\pm .3$ n=4	1.46 $\pm .003$ n=25	1.32 $\pm .001$ n=20	24.14 $\pm .04$ n=11	23.95 $\pm .05$ n=21

* ACTIVITY IS EXPRESSED AS MOLES P_i /MG PROTEIN/HR

** Z7:12AC - Z-7-DODECEN-1-OL ACETATE
12AC - DODECAN-1-OL ACETATE

TABLE II

PERTURBATION OF $\text{Na}^+ - \text{K}^+$ ATPASE ACTIVITY* IN I. NL. ANTENNAL SUBCELLULAR PARTICLE PREPARATIONS
BY PHEROMONE ODORANTS

MOLAR CONC.	MALE B FRACTION		FEMALE B FRACTION		MALE C FRACTION		FEMALE C FRACTION
	Z7:12AC**	12AC**	Z7:12AC	12AC	Z7:12AC	12AC	Z7:12AC
0	7.1±0.6 n=4	7.1±0.6 n=4	11.2±0.4 n=10	11.2±0.4 n=10	33.1±0.9 n=7	33.1±0.9 n=7	26.7±0.9 n=3
1X10 ⁻⁴	3.5(-51%)	3.0(-58%)	4.8(-57%) ±0.2 n=2	4.3(-62%)	16.2(-51%) ±1.4 n=3	10.9(-67%)	13.3(-50%) ±0.2 n=2
5X10 ⁻⁵	3.8(-46%)		6.7(-40%) ±0.4 n=2		18.9(-43%) ±1.7 n=3		19.1(-28%) ±1.6 n=4
1X10 ⁻⁵	7.7(8%)	7.6(7%)	11.0(-2%)		30.1(-9%) ±2.4 n=3	31.5(-5%)	23.7(-11%) ±1.2 n=4
5X10 ⁻⁶	8.5(20%)		11.1(-1%) ±0.05 n=3				
1X10 ⁻⁶	7.5(6%)	8.2(15%)	10.5(-6%) ±0.26 n=4	10.8(-4%)	31.9(-4%) ±3.2 n=2		26.5(-1%) ±0.7 n=3
5X10 ⁻⁷	6.5(-8%)		10.8(-4%) ±0.42 n=2				
5X10 ⁻⁷	7.4(4%)	7.1(0%)	10.3(-8%) ±0.85 n=3		33.4(1%) ±2.0 n=3		
1X10 ⁻⁸	7.6(7%)		10.6(-5%)		30.9(-7%) ±0.3 n=2		23.2(-13%)
1X10 ⁻⁹	8.9(25%) ±0.1 n=2	7.8(10%)		11.0(-2%)	34.0(3%) ±1.5 n=3	30.2(-9%)	
5X10 ⁻¹⁰	7.1(0%) ±0.4 n=2		11.3(1%)				25.8(-3%) ±1.2 n=2

* ACTIVITIES ARE CALCULATED AS $\mu\text{MOLES PI/MG}^{-1}$ PROTEIN/ HR^{-1}

** Z7:12AC = (7)-7-Nonadecen-1-ol Acetate

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