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Short Note

Effects of LAS on the Membrane Potential of Gill Epithelium in Young Tigerperch (*Terapon jarbua*)

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Dar-Yi Wang and Bao-Quey Huang (1994) Effects of LAS on the membrane potential of gill epithelium in young tigerperch (*Terapon jarbua*). *Zoological Studies* **33**(2): 170-173. In order to investigate the effects of LAS (Linear Alkylbenzene Sulfonate) on gill epithelium membrane potential, young tigerperch (*Terapon jarbua*), body lengths ranging between 57.5 and 67.5 mm, were kept in different concentrations of LAS for acute toxicity study. Intracellular recording results demonstrated that membrane potential shifted from -6.7 ± 1.4 mV ($n = 75$) to -5.1 ± 1.1 mV ($n = 63$) and -3.9 ± 1.1 mV ($n = 65$) when the LAS concentrations were changed by 5 ppm and 10 ppm, respectively. The decreasing membrane potential results in this study imply that LAS may lead to gill epithelium depolarization.

Key words: LAS, Intracellular recording, Estuarine teleost.

The tigerperch (*Terapon jarbua*) is a medium-sized edible fish and is recognized as having mariculture prospective in Taiwan (Liao 1988). The fish inhabit most of the estuarine waters of Taiwan where they feed and thrive during early developmental stages (Miu et al. 1990).

Linear Alkylbenzene Sulfonate (LAS) is a synthetic detergents widely used for household purposes in Taiwan (Chen et al. 1992). The primary injury inflicted by LAS is generally accepted as respiration difficulties and consequential death for many fishes (Zaccone et al. 1985, Roy 1988, Wang and Huang 1992). Histopathological investigation of LAS effects have shown gill damage and major microscopic gill epithelium destruction (Fukuda 1983, Misra et al. 1985, Wang and Huang 1992).

It has been found that detergents had similar effects on lipid peroxidation as local anesthetics, i.e. the inhibition of lipid peroxidation (Kihlstrom and Salminen 1991). Another effect these anesthetics have on the cell membrane is the block of ion fluxes (Baumgarten et al. 1991). In order to fully investigate the possible mechanism of LAS effects, it would be necessary to determine whether or not LAS detergent have the same effect on ion fluxes as the anesthetic does. In this study, we record the membrane potentials of gill epithelium in young tigerperch, and report the effects of the detergent, LAS, on the membrane potential.

Materials and Methods—Young tigerperch (*Terapon jarbua*) from the northeast Taiwan coast, ranging in body length from 57.5 mm to 67.5 mm, were kept in seawater aquarium; pH 7.91-7.99, salinity 35‰, water temperature 27-28°C. Subject fish were placed in a well aerated tank with various concentrations of LAS (Linear Alkylbenzene Sulfonate, Sigma, Co.) for 5 minutes; the fish was then sacrificed and its gill removed. The isolated gill was placed in an oxygen supplied recording chamber. Recordings were made from the outermost epitheium tissue.

Microelectrodes were prepared by drawing a micropipette of borosilicate glass (1 mm o.d. fiber filled, AM Systems Co. U.S.A.) on a puller (Narishige, PD-5, Japan). These microelectrodes were back filled with filtered 2.5 M KCl. The tip resistance of the microelectrodes, measured by a microprobe system (Nihon Kohden, JE-802J), ranging from 40-60 MΩ.

Figure 1 illustrates the equipment for response amplification, display, and recording. Responses were simultaneously fed into a preamplifier (MEZ 8201, Nihon-Kohden, Japan) and stored on magnetic tape with an FM data recorder (Sony DTE-77ES). Results were also charted (BD40 Kipp and Zonen, Dutch). A micromanipulator (Campden Instrument LTD, London) was used to position microelectrode with the gill epithelium.

Results and Discussion—Intracellular recordings of the LAS effects on gill epithelium membrane potential in young tigerperch are shown in Fig. 2. In our study, control group (Table 1) values ranged from -4.0 mV to -11.0 mV (-6.7 ± 1.4 mV, $n = 75$). In the control group (Fig. 3A), the membrane potential of the gill epithelium fluctuated; but, appeared distributed about a single mean (-6.7 mV). This indicates that there is only a single cell population (Huang 1986, Zadunaisky et al. 1988). Epithelium cells form the main part of lamella surface while chloride and mucous cells are only located within the deeper filament area (Laurent 1984, Payan et al. 1984, Jagoe and Haines 1990). In our experiment, intracellular recordings were obtained only from the most superficial gill which assured that the cell population was of a single type.

When recordings were made of the gills treated with 5 ppm LAS, the membrane potential range changed to -3.0 mV and -7.0 mV (-5.1 ± 1.1 mV, $n = 63$, Table 1). Following

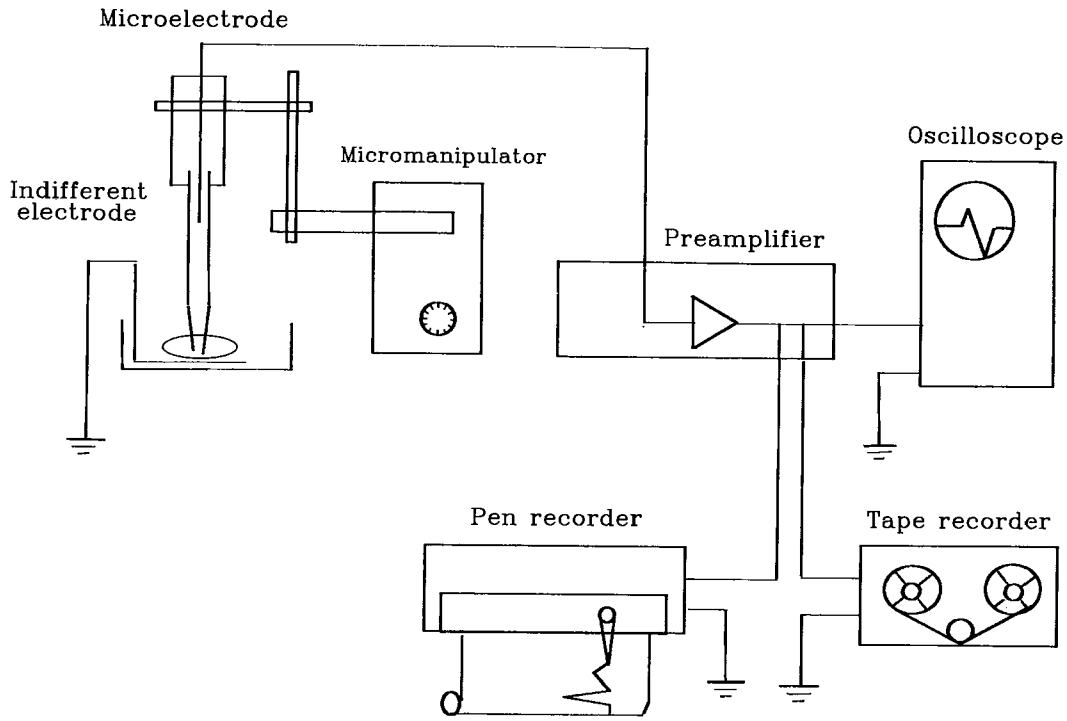

Fig. 1. Intracellular recording of gill epithelium equipment schematic.

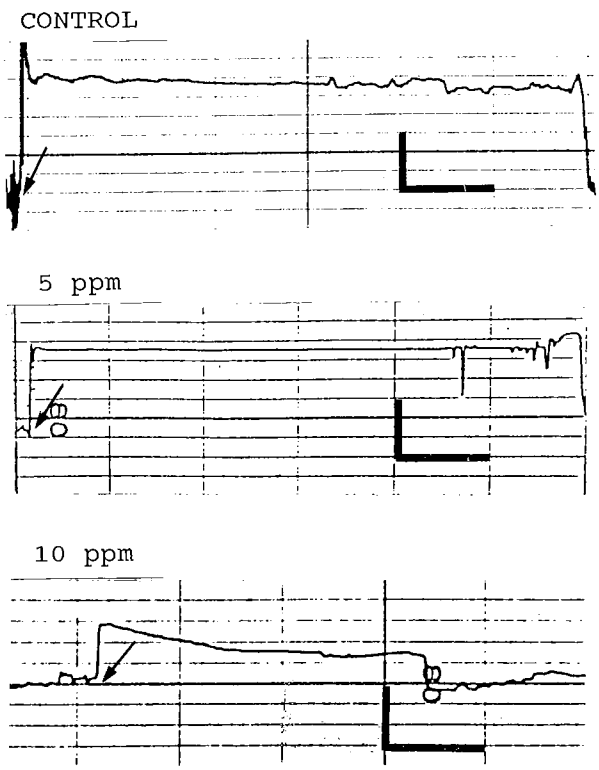

Fig. 2. Recording traces of gill epithelium membrane potential after treated with various concentration of LAS. Arrowheads, recording baseline (0 mV).
Calibration: 3 mV, 5 sec.

Table 1. Mean and standard deviations of membrane potential of gill epithelium after treatment of LAS. a.b.c mark the significant difference examined by *t*-test ($p < 0.05$)

LAS Conc.	Membrane potential (mV)	*t*-test
0 ppm	-6.7 ± 1.4 (n = 75)	a
5 ppm	-5.1 ± 1.1 (n = 63)	b
10 ppm	-3.9 ± 1.1 (n = 65)	c

application of 10 ppm LAS, the potential range shifted to −2.0 mV and −6.0 mV (−3.9 ± 1.1 mV, n = 65, Table 1). The switch to solution containing 5 or 10 ppm LAS (Figs. 3B, C), resulted in a declining shift of membrane potential distribution, −5.1 mV and −3.9 mV, respectively. The depolarizing effects of LAS on the membrane potentials of the gill epithelium were significant ($p < 0.05$, Table 1). The results obtained from the present work revealed that LAS causes gill epithelium cells depolarization.

Kihlstrom and Salmimen (1991) suggested that some local anesthetics (e.g. cocaine, lidocaine) and detergents (e.g. Sodium dodecyl sulphate, Sodium deoxycholate) by causing a rearrangement of membrane lipid bilayer had very similar effects on lipid peroxidation. It is widely-accepted that the principal mechanism of local anesthetics is thought to be an all-or-none Na^+ channel block (Frazier et al. 1970, Baumgarten et al. 1991). Local anesthesia is caused by a slight membrane

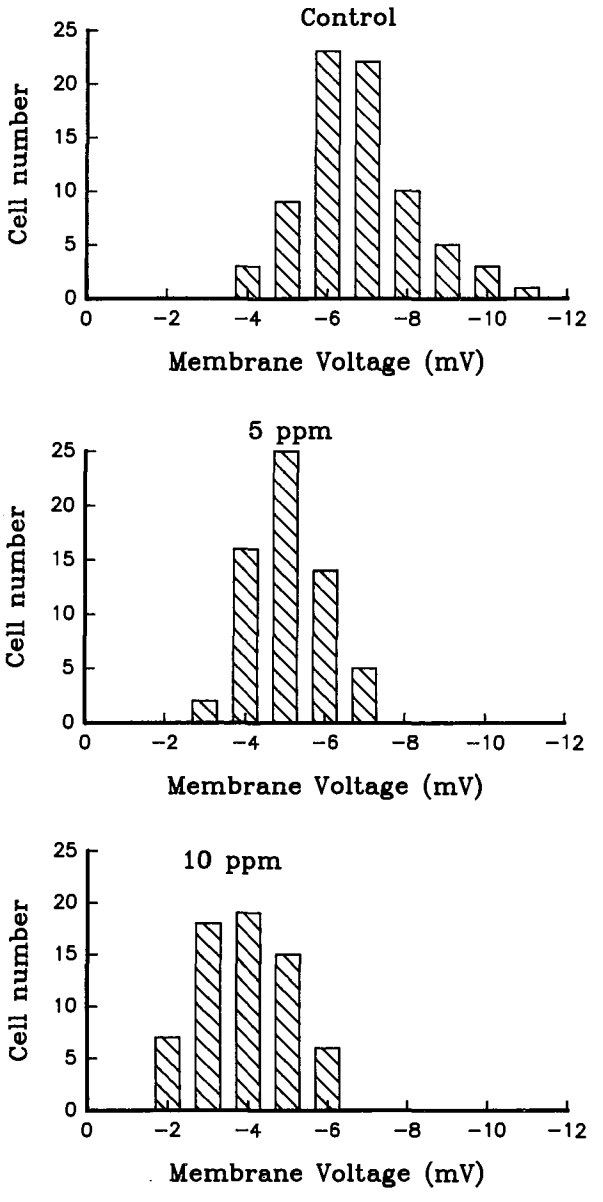

Fig. 3. Effect of different concentraitons of LAS (control, 5 ppm, 10 ppm) on membrane potential of the gill epithelium. Note the distribution are shifted.

depolarization (Ritchie and Greene 1992). Depolarization in the cell membrane of the gill epithelium from exposure to LAS may be explained with occurring of some general changes in membrane characteristics. Thus the question of LAS detergent effects exertion in lipid peroxidation by membrane structural reorganization inducement (as in local anesthetics), resulting in a Na^+ channel block and responding depolarization remains for further investigation.

In conclusion, intracellular measurements from the gill epithelium of young tigerperch present a single cell type. The studied cells respond to LAS in vivo treatment with lowered membrane potentials i.e. depolarization.

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清潔劑(LAS)對鰓上皮細胞膜電位之影響

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實驗以玻璃顯微電極(glass microelectrode technique)，進行單一細胞之胞內記錄(intracellular recording)，以探討清潔劑LAS (linear alkylbenzene sulfonate)對花身雞魚(*Terapon jarbua*)稚魚之鰓部上皮細胞膜電位之影響。實驗結果顯示，在對照組75個成功胞內記錄中，膜電位之頻率分佈呈現單峰常態分佈，平均膜電位爲−6.7±1.4mV (n=75)，顯示屬單一群的皮膜細胞，而活體魚經過LAS (5ppm、10ppm)急性毒處理5分鐘後，膜電位分別轉變爲−5.1±1.1mV (n=63)及−3.9±1.1mV (n=65)。經t-test檢定，顯示此一濃度LAS處理確與對照組呈現顯著差異($p<0.05$)；膜電位的改變顯示LAS可能作用於鰓部之上皮細胞，使其對離子之通透性改變而產生去極化(depolarization)的現象。

關鍵詞：直鏈式烷基苯磺酸鹽(LAS)，胞內記錄，河口硬骨魚。