

## ETHANOL EFFECTS ON THE BIOELECTRIC ACTIVITY OF THE TOAD SKIN

Efectos del Etanol sobre la actividad bioeléctrica de la piel de sapo.

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### RESUMEN

Se estudió el efecto de etanol 0.043-0.284 M sobre la diferencia de potencial (d. p.) y la corriente de corto-circuito (c. c. c.) de la piel de dos especies de sapo. El etanol alteró ambos parámetros probablemente a través de la estimulación de receptores alfa o beta. Este efecto desapareció al bloquear la vía glicolítica mediante iodoacetato o por falta de calcio. Ouabaina en concentraciones que bloquean la ATPasa  $\text{Na}^+\text{K}^+$  produjo una disminución marcada de la c. c. c. La perfusión con etanol disminuyó en forma reversible la respuesta de glándulas mucosas a noradrenalina. Estos resultados sugieren que el etanol (0.043 M) reduciría la actividad mioepitelial y que las concentraciones altas de etanol (0.284 M) posiblemente liberen noradrenalina desde las terminaciones nerviosas simpáticas pero aparentemente no inhibirían la ATPasa  $\text{Na}^+\text{K}^+$ . Para inhibir esta enzima son necesarias concentraciones mayores de etanol (0.4 M).

### ABSTRACT

The effect of 0.043-0.284 M ethanol on the potential difference (p. d.) and on the short-circuit current (s. c. c.) of the skin of two species of toad was studied. Ethanol altered both parameters probably through either alpha or beta receptor stimulation. Blockade of the glycolytic pathway by iodoacetate or lack of calcium abolished this effect. Ouabain in concentrations which block  $\text{Na}^+\text{K}^+$ -activated ATPase markedly reduced the s. c. c. Perfusion with ethanol reversibly reduced the response of mucous secreting glands to noradrenaline. These results suggest that 0.043M ethanol may affect myoepithelial activity and that high concentrations of ethanol (0.284 M) possibly release noradrenaline from sympathetic nerve terminals but apparently do not inhibit  $\text{Na}^+\text{K}^+$ -activated ATPase. Higher concentrations (0.4 M) of ethanol were necessary to produce inhibition of this enzyme.

Keywords: Amphibia. Ethanol action. Bioelectric activity. Physiology.

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## INTRODUCTION

Israel, Kalant and Laufer (1965) reported that ethanol causes an inhibition of  $\text{Na}^+$ - $\text{K}^+$ -activated ATPase and that this effect has also been found in the skin of *Rana pipiens*. The short-circuit current (s.c.c.) across the skin, which is a function of the net transport of sodium, decreases in the presence of ethanol (Israel et al., 1963).

Concha et al. (1970) proposed that stimulation of adrenergic receptors by ethanol or by noradrenaline might increase the p.d. and the s.c.c. by enhancing the rate of glycogenolysis. Morel and Jard (1971) attributed the reduction of these responses to inhibition of adenylyl cyclase due to stimulation of alpha receptors; however, a decrease in permeability has also been postulated (Moore, 1966; González et al., 1969).

The experiments described in this paper were undertaken to study the effect of ethanol on the potential difference (p.d.), on the s.c.c. and on the bioelectric response to noradrenaline, of the isolated skins of two species of Chilean toad, *Bufo arunco* and *Pleurodema thaul*.

We suggest that the effect of ethanol in the concentration range used in these experiments is not limited to the inhibition of the transport mechanism as postulated by Israel and Kalant (1965). The results indicate that ethanol probably elicits permeability changes and releases noradrenaline from the nerve terminals. This catecholamine then acts at alpha or beta receptor sites as postulated by González et al. (1969).

## METHODS

## 1) ANIMALS.

Experiments were performed at room temperature (18–23°C) on fragments of abdominal skin removed from 29 female *Bufo* toads (70–130 g) and on skin glands of 21 *Pleurodema thaul* toads (7–15 g) during the summer and autumn months of December to April. The toads were either killed immediately after they were caught or were kept in a humid environment at 18° to 23°C and fed on sow bugs (*Oniscus asellus*).

The glands can be seen on all parts of the surface of *Pleurodema* under an ocular micrometer (Noble et al., 1944); they are mucous in character, usually spherical in shape, and are 51–102  $\mu\text{m}$  in diameter. They open onto the epidermal surface through a stoma 17–34  $\mu\text{m}$  in diameter.

## 2) SOLUTIONS.

Table I gives the composition of the toad Ringer and of the other solutions used in this investigation. All of the solutions were buffered with  $\text{Na}_2\text{HPO}_4$  at pH 7.4 and gassed with  $\text{O}_2$ .

## 3) MEASUREMENTS OF P.D. AND S.C.C. OF ISOLATED TOAD SKINS.

Records of the p.d. were made in the experiments on *Bufo* skins. The equipment for measuring s.c.c. was mounted in later experiments using *Pleurodema* skins. The skins were dissected from pithed toads and mounted vertically as membranes separating two halves of a perspex chamber, constructed so that 1.33  $\text{cm}^2$  of skin surface was exposed to 4 ml

toad Ringer on each side. The p.d. across the skin was measured by means of Ag-AgCl electrodes placed in the bathing medium (fig. 1). The electrodes were connected to a Grass model 5P1 d.c. preamplifier, led to a driver amplifier and the potentials recorded with a Grass model 5C direct writing oscillograph.

The s.c.c. was monitored with platinum electrodes placed at 5 mm distance from the skin, connected to a microammeter having a scale reading of 50 microamperes provided with shunts to read a full scale of 300  $\mu$ A, and to a clamping system built in the laboratory which automatically adjusted the s.c.c. to keep the p.d. of the skin at zero. The output was led to a Grass model 5C polygraph amplifier and the record of the current was displayed on paper through a Grass electromagnetic oscillograph.

After the skins had been mounted in the apparatus, the p.d. or the s.c.c. were measured continuously. When it was observed that both measurements were constant (about 30–40 min), drugs were added to the medium bathing the serosal surface.

#### 4) RECORDING OF ELECTRICAL ACTIVITY OF SINGLE MUCOUS GLANDS.

The skin was mounted between two compartments of a perspex chamber with the outer side facing upward. The serosal surface of the skin was in contact with toad Ringer, which was perfused through the chamber at a rate of 1 ml/min, and was bubbled with O<sub>2</sub>. The epidermal side was in air with an exposed surface of 2.27 cm<sup>2</sup>.

The electrical changes in the glands were recorded with glass micropipettes which were introduced through the stoma of the gland. They were drawn on a puller and filled with isotonic Ringer. Their diameter was 4–6  $\mu$ m and their resistance was 2–5 megohms. Observations were rejected if the micropipette characteristics changed after impalement. They were connected to a Grass P<sub>16</sub> microelectrode d.c. preamplifier through a Ag-AgCl wire. The amplifier potentials were led to a Grass driver amplifier and recorded on paper with a Grass model 5C oscillograph. The reference electrode was Ag-AgCl. During the experiments, which lasted 3–4 hours, the measured p.d. remained stable.

Glandular secretion was estimated by microscopic examination of the size and number of droplets extruded through the stoma of individual glands.

#### 5) DRUGS.

The following drugs were used: 1-noradrenaline bitartrate (Instituto Bioquímico Beta), Pronethalol, Dibenamine and Ethanol (Merck), Reserpine (Silesia), Ciba Compound 39-089-Ba, Ouabain (Calbiochem) and Iodo acetate (Sigma). All drugs were added to the perfusion fluid in a volume of 0.1 ml Ringer to give the final concentrations mentioned in the text. Fresh solutions were prepared daily from the stock solutions.

In isolated skin experiments, ethanol was added to the medium bathing the serosal surface of the skin in amounts sufficient to produce final concentrations ranging from 0.192 to 0.284 M (0.88–1.3 g%). In the single gland experiments, all drugs were given in Ringer into a rubber cannula at a distance of 6 cm from the micropipette. In some of the single

gland experiments 0.043 M ethanol Ringer was used to replace toad Ringer.

Control injections of equal volumes of Ringer were always made.

## RESULTS

### EXPERIMENTS ON ISOLATED SKINS OF BUFO ARUNCO.

a) *Effect of ethanol.* In 16 of 27 experiments, 0.192 M ethanol induced a significant ( $P < 0.01$ ) increase in p.d., which was variable in magnitude and of short duration, followed by a sustained and significant ( $P < 0.05$ ) depolarization (fig. 2). In 18 of 27 experiments only depolarization was elicited and in 3 cases an increase in p.d. was the sole response.

b) *Effect of pronethalol on the action of ethanol.* When pronethalol ( $1.7 \times 10^{-6}$  M) was applied, a significant ( $P < 0.001$ ) increase in p.d. was observed in 4 experiments. On adding pronethalol to the medium bathing the serosal surface the effect of 0.192 M ethanol was abolished. Fig. 3 shows that a predominantly depolarizing effect of ethanol was blocked by pronethalol.

c) *Effect of dibenamine on the action of ethanol.* Dibenamine ( $1.8 \times 10^{-6}$  M) produced a significant ( $P < 0.01$ ) reduction of the p.d. in 3 experiments. On addition of dibenamine to the serosal surface of the skin, the hyperpolarizing phase of the response to 0.192 M ethanol was abolished; only a sustained depolarization was elicited (fig. 4).

### EXPERIMENTS ON ISOLATED SKINS OF PLEURODEMA THAUL.

a) *Effect of ethanol.* Ethanol (0.192 M) increased the s.c.c. of the skins. In 7 of 12 experiments the response consisted of more than one component; in most cases a rapid initial waveform of 4 min duration was followed by a slow response which declined to basal values in 10–12 min. In some of these experiments the rapid component was much shorter and in 5 of 12 cases only a slow waveform was recorded. For both groups, the change in current was significant ( $P < 0.01$ ).

In these experiments ethanol also induced a notable decrease of the glandular secretion evoked by noradrenaline.

b) *Effect of compound Ciba 39-089-Ba on the action of ethanol.* This compound (15  $\mu$ g/ml) practically abolished the effect of ethanol on the skin of Pleurodema in 6 experiments (fig. 5).

c) *Effect of dibenamine on the action of ethanol.* Dibenamine ( $1.8 \times 10^{-6}$  M) did not block the effect of ethanol or the effect of  $10^{-7}$  M noradrenaline in 4 experiments.

These experiments show that dibenamine increases the p.d. across the isolated skin of Pleurodema (fig. 6).

d) *Effect of reserpine on the action of ethanol.* The skins of a control group (fig. 7) of 5 amphibians showed the characteristic responses to 0.192 M ethanol. In contrast, the s.c.c. of the skins of 5 toads injected intraperitoneally with 10 mg/kg reserpine 24 hours prior to the experiment, was unchanged by 0.192 M ethanol (fig. 7A) and the response to 0.284 M ethanol was notably reduced (fig. 7B).

The magnitude of the response to noradrenaline of the skins of reserpinized animals was significantly ( $P < 0.01$ ) increased compared to control skins (fig. 8) and the time course of the response was considerably lengthened.

e) *Effect of iodoacetate on the action of ethanol and of noradrenaline.* When  $10^{-3}$  M iodoacetate was applied to the serosal surface of the skin, the response to 0.192 M ethanol and to  $1 \times 10^{-7}$  M noradrenaline was notably decreased in 4 cases (fig. 9).

f) *Effect of the absence of calcium in the bathing medium.* When calcium was removed from the bathing medium, the response of the skin to 0.192 M ethanol was abolished in 4 experiments (figs. 10). The response to  $1 \times 10^{-7}$  M noradrenaline was unaffected. The re-introduction of calcium to the medium restored the response to ethanol.

g) *Effect of ouabain.* The addition of  $10^{-6}$  M ouabain to the serosal surface produced a notable reduction of the s.c.c. of the skin of Pleurodema in 5 experiments (fig. 11). Fig 12 illustrates skins exposed to  $10^{-6}$  M ouabain, which showed a progressive reduction of the s.c.c. and a marked decrease of the response to noradrenaline in 3 experiments.

There is considerable variation in the ability of different alcohols to release noradrenaline. At high concentration (0.4 M) methanol seems to be the most effective, whereas both ethanol and propanol reduce the p.d. of the skin (fig. 13).

#### EXPERIMENTS ON INDIVIDUAL SKIN GLANDS OF PLEURODEMA THAUL.

*The effect of perfusion with 0.043 M ethanol in Ringer solution on the response to noradrenaline of the single glands.* The responses of individual skin glands to  $10^{-7}$  M noradrenaline could be elicited repeatedly in 9 experiments and were always identical. These responses consisted of hyperpolarization waveforms of different latencies. In 6 experiments a rapid component was followed by a slow response.

When the preparation was perfused with 0.043 M ethanol-Ringer instead of the normal toad Ringer, there was a very slight decrease in the p.d., and the application of  $10^{-7}$  M noradrenaline produced a sudden decrease in the magnitude of the rapid component whereas only a slight diminution could be observed in the slow waveform (fig. 14). The partial blockade induced by perfusion with ethanol-Ringer could be quickly reversed when perfusion with normal Ringer was re-established and the resting potential showed a tendency to increase.

#### DISCUSSION

The present findings are consistent with the hypothesis that ethanol releases noradrenaline from nerve terminals, thus activating the alpha or the beta receptors of the skin. The results with pronethalol suggest that beta blockade serves to unmask an alpha effect responsible for hyperpolarization; and the action of dibenamine seems to indicate that alpha blockade unmasks beta receptors which now exert a depolarizing effect (Morel and Jard, 1971; González et al., 1969). These experiments on the skin of *Bufo arunco*, which show that the events evoked by ethanol and

noradrenaline can be suppressed by alpha and beta receptor blockade, provide an alternative explanation of the results reported by Israel et al. (1965), which show that ethanol at concentrations similar to those used by us, should inhibit  $\text{Na}^+$ - $\text{K}^+$ -activated ATPase directly.

Since iodoacetate and fluoride block the effect of both noradrenaline and of ethanol (Concha et al., 1970) it is possible that stimulation of adrenergic receptors by ethanol or by noradrenaline increases the p.d. and the s.c.c. by enhancing the rate of glycogenolysis. The end result should be an augmentation of the active transport of sodium across the skin.

Further evidence in favour of this hypothesis is the observation that whereas ethanol has no effect on the skins of reperfused animals (fig. 7), the response of these skins to noradrenaline is increased due to the development of hypersensitivity (González et al., 1967).

The inhibition of the response to ethanol when the preparation is perfused with calcium-free Ringer would seem to indicate that this ion is indispensable for the release of noradrenaline from the nerve endings. Removal of calcium from the perfusion fluid does not affect the adrenergic receptors, since the response to noradrenaline under these conditions is not altered (fig. 10). It is well known that calcium is necessary for the release of chemical mediators, and the importance of its role in terminals such as the motor end-plate is fully established.

Perfusion of the skins of *Pleurodema* with 0.043 M ethanol-Ringer decreased the rapid component of the hyperpolarizing response of the skin glands to noradrenaline, but had only a slight effect on the slow component. The perfusion experiments on skin glands were performed with ethanol concentrations (0.043 M) which were similar to the weaker concentrations used by Israel and Kalant (1963), but were about 6 times weaker than the strongest concentrations (0.284 M) used in the experiments on blocks of skin. We consider that ethanol at this concentration has no effect on the release of noradrenaline, since no changes in the p.d. of the isolated skin can be elicited. The decreased response of the skin glands to noradrenaline could be explained as follows:

The effect of ethanol on the rapid component might be due to an increase in the electrical resistance of the skin brought about by a decrease in glandular secretion (González et al., 1969). This is the case in other tissues such as the giant axon of the squid, where ethanol decreases permeability (Moore, 1966). This decrease in permeability might explain the marked reduction of the rapid component produced by low concentrations of ethanol.

The very slight reduction of the slow component of the response induced by noradrenaline might be attributed to partial inhibition of the active transport of sodium due to a decrease in permeability which reduces the quantity of sodium available to the pump. The slow component disappears after addition of  $10^{-6}$  M ouabain to isolated skin (fig. 12), which points to inhibition of active transport of sodium. Fig 15 shows a possible mechanism of action of ethanol on the toad skin. The results of this work suggest that ethanol in relatively high concentrations (0.192-0.284 M) acts by releasing noradrenaline from the nerve endings. If noradrenaline then activates the adenyl cyclase system, the rate of the

glycogenolytic pathway should increase and thus enhance sodium transport due to stimulation of the respiratory cycle which generates ATP.

The reduction in s.c.c. and in p.d. by ethanol, observed in some experiments, might be explained as an inhibition of adenylyl cyclase due to stimulation of alpha receptors, a hypothesis proposed by Morel and Jard in 1971.

We have as yet no direct evidence for the release of catecholamines by ethanol and it has not been possible to measure adenylyl cyclase activation. Further work is being planned in this field in the laboratory.

A particularly interesting feature of these experiments is the species difference in the activity induced by stimulation of alpha and beta adrenergic receptors. In *Bufo arunco*, alpha adrenergic stimulation increases the p.d. and s.c.c. across the skin, and beta adrenergic stimulation has the opposite effect. In *Pleurodema thaul* beta adrenergic stimulation increases the p.d. and the s.c.c. and alpha adrenergic stimulation reduces both parameters.

The results of the present experiments show that the mechanism of action of ethanol at low concentration (0.043 M) is different from the mechanism of action of ouabain. The decrease of the slow response to noradrenaline of isolated *Pleurodema* skins exposed to ouabain (fig. 12) suggests that the slow component may be due to active transport of sodium.

We consider that the concentrations of ethanol used in these experiments (0.043–0.284 M) are too low to block Na<sup>+</sup>K<sup>+</sup>-activated ATPase and it is very doubtful that ethanol acts by direct stimulation or inhibition of adenylyl cyclase. However, the depressant action of ethanol and propranolol at high concentration (0.4 M) might be explained as a direct inhibition of Na<sup>+</sup>K<sup>+</sup>-activated ATPase (fig. 13).

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TABLE I.

Composition of solutions (mM). The pH of each solution was 7.4

	TOAD RINGER	ETHANOL RINGER	CALCIUM-FREE RINGER
NaCl	112.00	112.00	114.00
KCl	1.90	1.90	1.90
CaCl <sub>2</sub>	2.00	2.00	—
NaHCO <sub>3</sub>	2.30	2.30	2.30
Glucose	11.00	11.00	11.00
NaHPO <sub>4</sub>	0.04	0.04	0.04
Ethanol	—	0.043	—

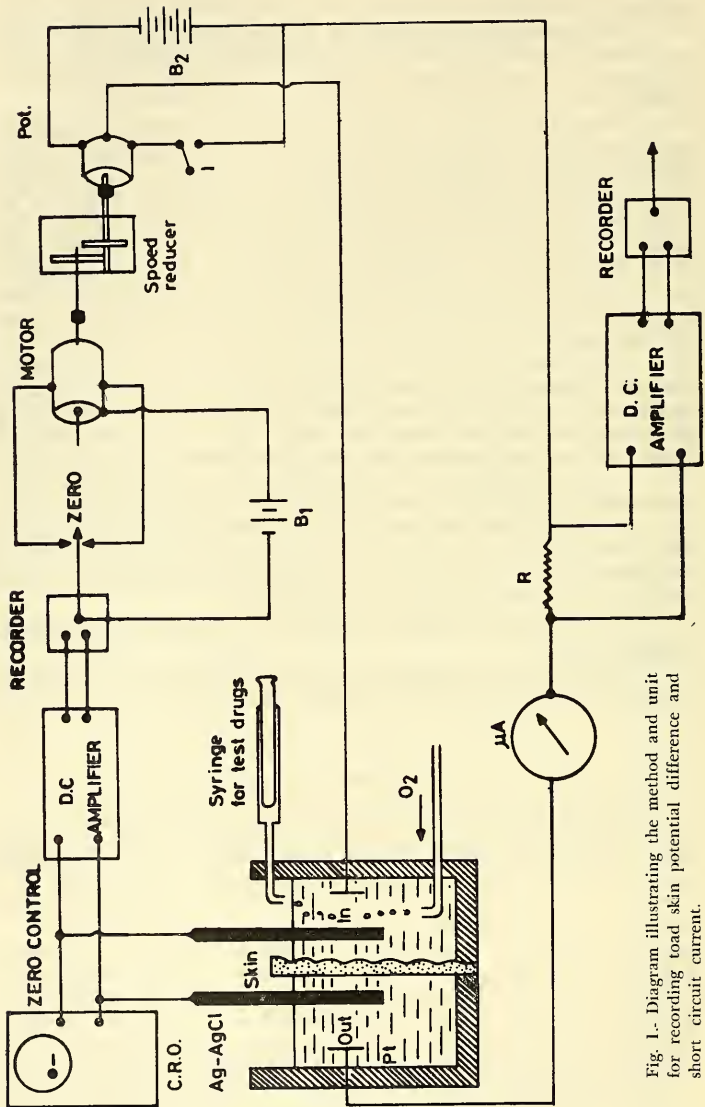


Fig. 1.- Diagram illustrating the method and unit for recording toad skin potential difference and short circuit current.

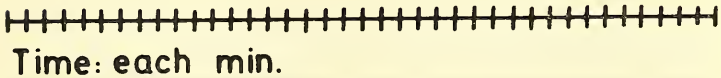
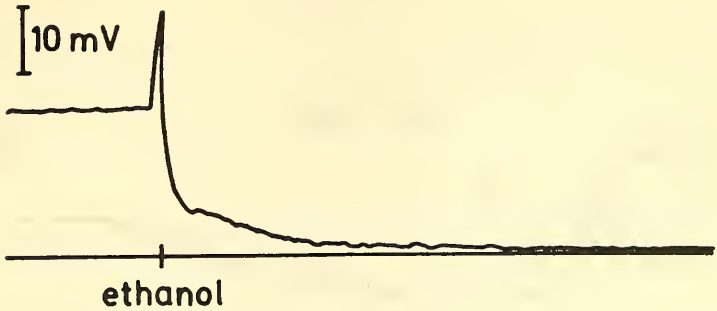


Fig. 2.- Time courses of the responses in the p.d. of the skin of *Bufo arunco* to ethanol. Toad Ringer solution. Temp. 20°C. Ethanol was added to the serosal surface of the skin to give a final concentration of 0.192 M. Resting potential = 42 mV. The middle trace in this and all the following figures is a reference line and does not indicate zero potential.

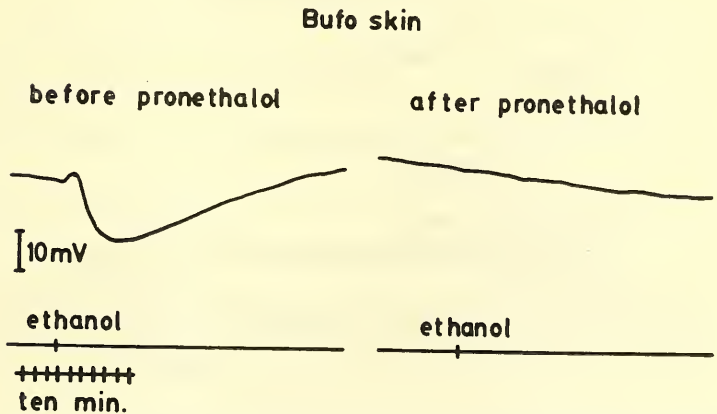


Fig. 3.- Comparison of the responses in the p.d. of the skin of *Bufo arunco* to 0.192 M ethanol before (left) and after (right) addition of  $1.7 \times 10^{-6}$  M pronethalol. Toad Ringer solution. Temp. 22°C Both drugs were added to the serosal surface of the skin to give the final concentrations mentioned above.

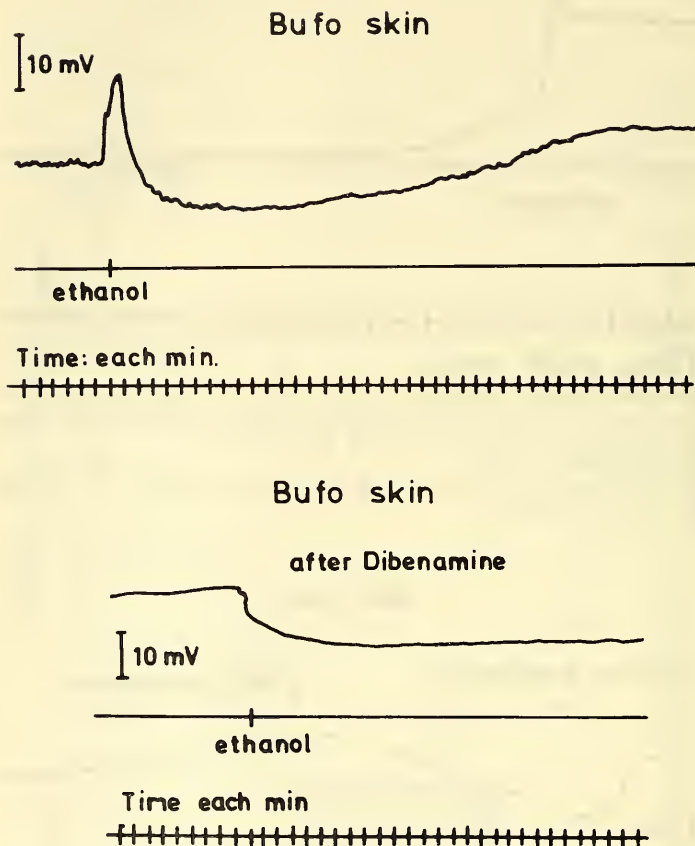


Fig. 4.- Comparison of the responses in the p.d. of the skin of *Bufo arunco* to 0.192 M ethanol, before ( above) and after (below) addition of  $1.8 \times 10^{-6}$  M dibenamine. Toad Ringer solution. Temp. 18°C Both drugs were added to the serosal surface of the skin to give the final concentrations mentioned above. Resting potential = 36 mV.

Pleuro skin

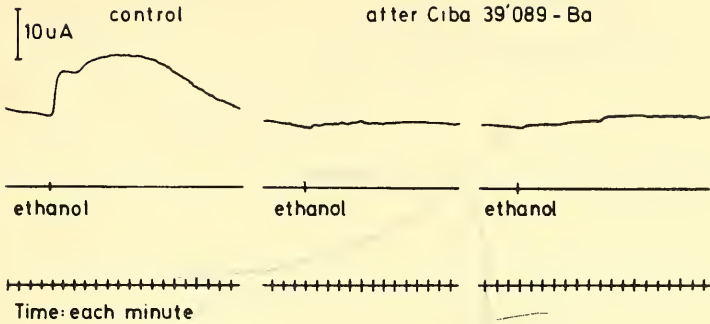


Fig. 5.- The inhibitory effect of 15 ug/ml Ciba compound 39-089-Ba on the responses in the s.c.c. of the skin of *Pleurodema thaul* to 0.192 M ethanol. Toad Ringer solution. Temp. 22°C. Both drugs were added to the serosal surface of the skin to give the final concentrations mentioned above. *Left*: control skins showed rapid and slow responses to ethanol. The responses disappeared at 20 min (*centre*) and at 40 min (*right*) after addition of Ciba compound. Base line current = 48 uA. cm.<sup>2</sup>.

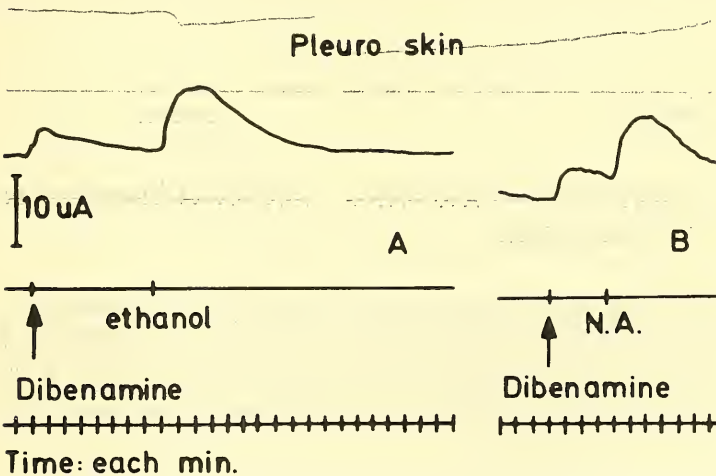


Fig. 6.- Comparison of the responses in the s.c.c. of the skin of *Pleurodema thaul* to ethanol and to noradrenaline, in the presence of dibenamine. Toad Ringer solution. Temp. 18°C. The drugs were added to the serosal surface to give the final concentrations mentioned below. The effects of 0.192 M ethanol (A) and of  $10^{-7}$  M noradrenaline (B), were not altered in the presence of  $1.8 \times 10^{-6}$  M dibenamine. Base line current = 52 uA. cm.<sup>2</sup>.



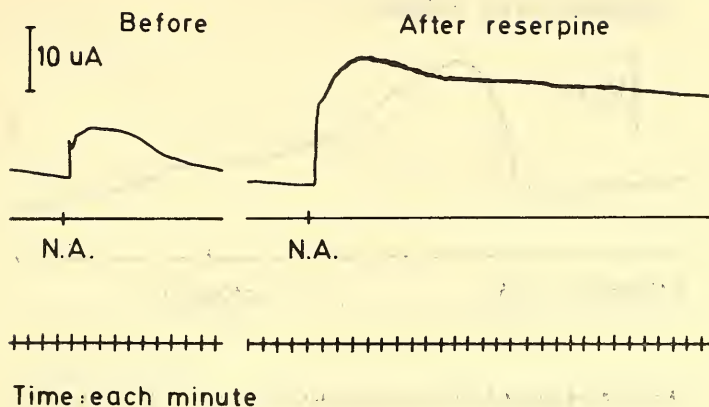


Fig. 8.- Effect of 10 mg/kg reserpine injected intraperitoneally 24 hours previously, on the response in the s.c.c. of the skin of *Pleurodema thaul* to noradrenaline. Toad Ringer solution. Temp. 22°C. Noradrenaline was added to the serosal surface of the skin to give a final concentration of  $10^{-7}$  M. Control skins (left) showed the rapid and slow responses of the skin to noradrenaline (base line current = 30 uA); on the right, the effect of noradrenaline is enhanced by reserpine (base line current = 26 uA. cm.<sup>2</sup>).

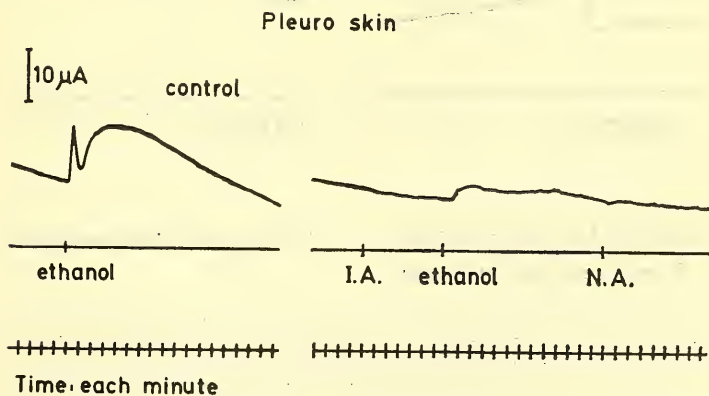


Fig. 9.- The inhibitory effect of iodoacetate on the response in the s.c.c. of the skin of *Pleurodema thaul* to ethanol and to noradrenaline. Toad Ringer solution. Temp. 18°C. All drugs were added to the serosal surface of the skin to give the final concentrations mentioned below. Left. Effect of 0.192 M ethanol (base line current = 45 uA). Right. Effect of 0.192 M ethanol and of  $10^{-7}$  M noradrenaline after addition of  $10^{-7}$  M iodoacetate (base line current = 40 uA. cm.<sup>2</sup>).

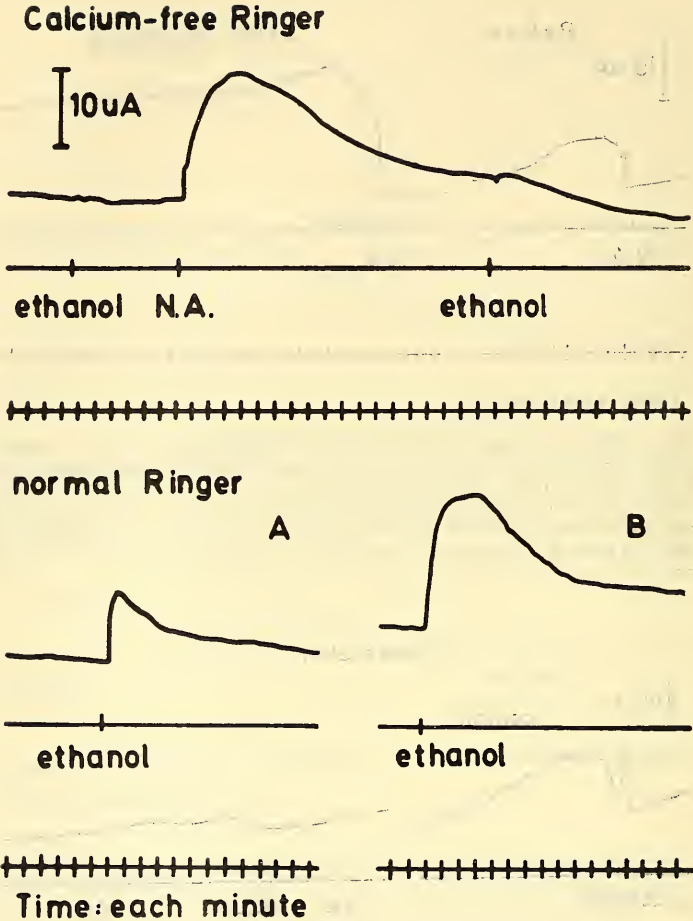


Fig. 10.- The effect of perfusion with calcium-free toad Ringer on the responses in the s.c.c. of the skin of *Pleurodema thaul* to ethanol and to noradrenaline. Both drugs were added to the serosal surface of the skin to give the final concentrations mentioned below. Temp. 19°C. *Above.* Calcium-free Ringer. There is a notable response in the s.c.c. to  $10^{-7}$  M noradrenaline, whereas no change is elicited on addition of 0.192 M ethanol. Base line current = 45 uA. cm.<sup>2</sup>. *Below.* Normal toad Ringer. Effect of 0.192 M ethanol a) 15 min and b) 20 min after calcium is restored to the perfusion fluid, N. A. = noradrenaline.

PLEURO SKIN

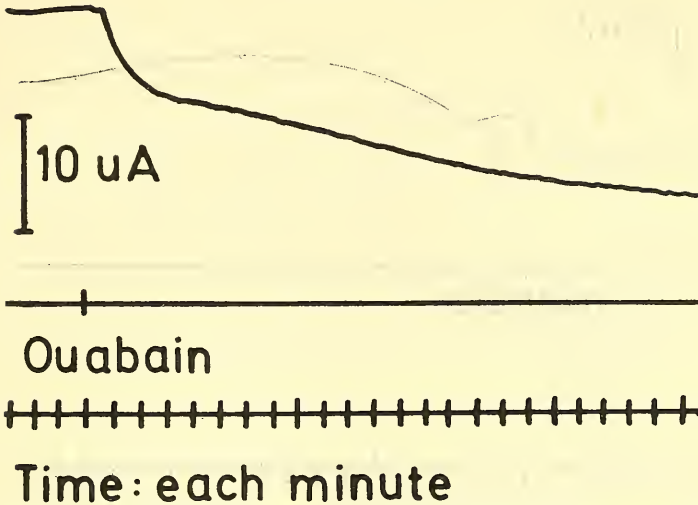


Fig. 11.- Time course of the response in the s.c.c. of the skin of *Pleurodema thaul* to ouabain. Toad Ringer solution. Temp. 189C. Ouabain was added to the serosal surface of the skin to give a final concentration of  $10^{-5}$  M. Base line current = 80 uA. cm.<sup>2</sup>.

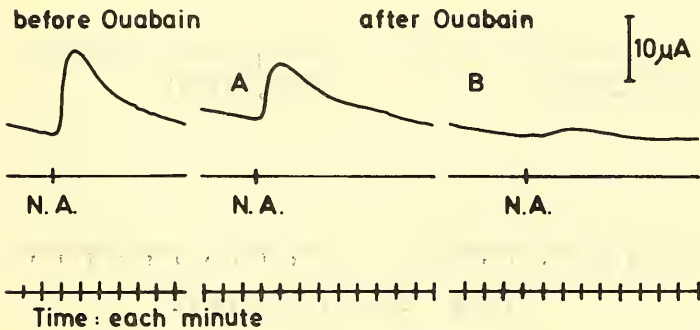


Fig. 12.- Time course of the responses in the s.c.c. of the skin of *Pleurodema thaul* to noradrenaline, before and after ouabain. Toad Ringer solution. Temp. 209C. All drugs were added to the serosal surface of the skin to give the final concentrations mentioned below. *Left.* Response to  $10^{-7}$  M nordrenaline. *Right.* Response to  $10^{-7}$  M noradrenaline a) 10 min and b) 20 min after addition of  $10^{-6}$  M ouabain. Base line current = 80 uA. cm.<sup>2</sup>.

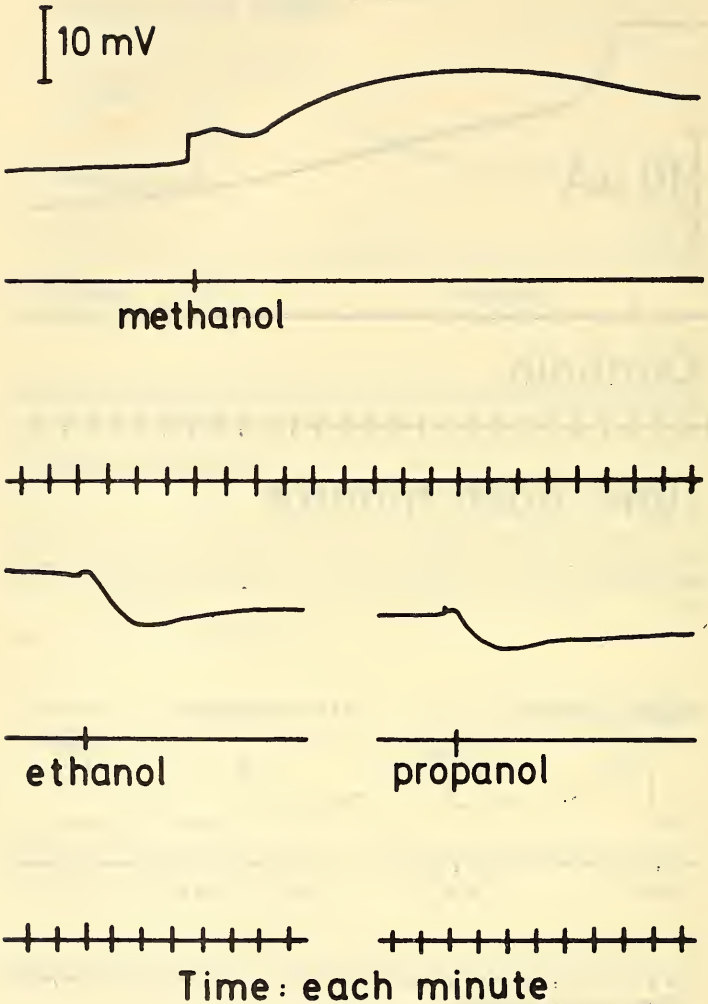


Fig. 13.- Time courses of the responses in the p.d. of the skins of *Pleurodema thaul* to high concentrations of several alcohols. Toad Ringer solution. Temp. 20°C. The alcohols were added to the serosal surface to give a final concentration of 0.4 M. *Above.* The response to methanol. *Below.* The response to ethanol and to propanol. Resting potential = 30 mV.

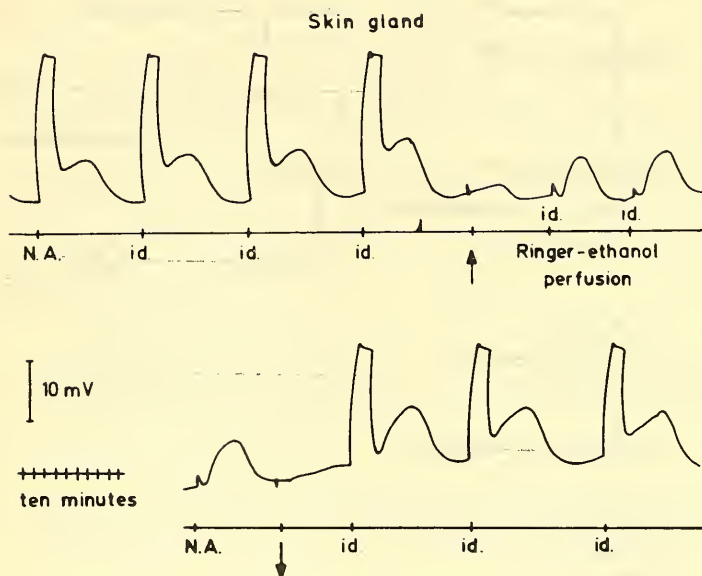


Fig. 14.- Time courses of the responses in the p.d. of a skin gland of *Pleurodema thaul* to repeated applications of noradrenaline, under conditions where normal toad Ringer solution was replaced by 0.043 M ethanol-Ringer. Temp. 209C. This record of a single gland was obtained by means of an intraglandular micropipette. Noradrenaline was added to the serosal surface of the skin to give a final concentrations of  $10^{-7}$  M. *Above.* Skin gland perfused with normal toad Ringer solution. The effect of repeated applications of noradrenaline consisted of a rapid\* and a slow increase of the p.d. At the arrow perfusion was changed to 0.043 M ethanol-Ringer and on addition of noradrenaline there was a sudden decrease in the magnitude of the rapid response. *Below.* At the arrow perfusion with normal toad Ringer was re-established and on addition of noradrenaline the responses of the skin glands were quickly restored. N. A. = noradrenaline. Resting potential = 29 mV. Note the steady increase in resting potential when perfusion with normal Ringer is restored.

\*The plateau of the rapid response is an artifact due to saturation of the movement of the recording pen.

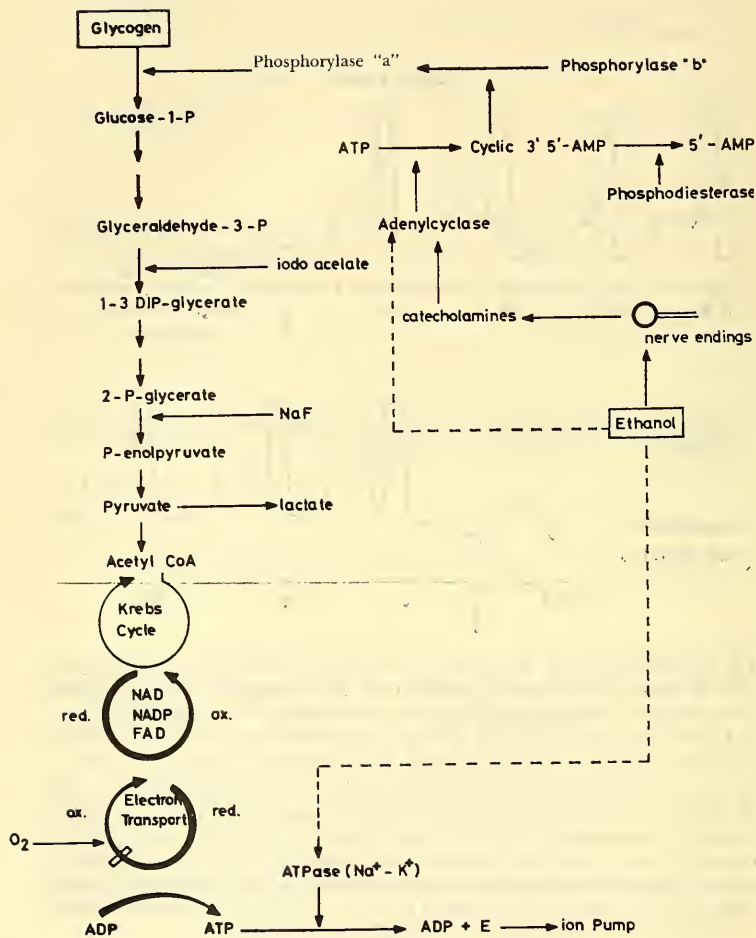


Fig. 15.- Summary of possible mechanisms of action of ethanol on the isolated skin of the toad. See text for details.

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