Statistical comparisons of steady potential responses

Time of Stimulus (sec)	Habituation (first 40 trials) vs. Habituation (last 40 trials)		Habituation (last 40 trials) vs. Pairing (first 40 trials)		Pairing (first 40 trials) vs. Extinction (first 40 trials)		Pairing (first 40 trials) vs. Extinction (last 40 trials)	
	t[a]	P[b]	t	P	t	P	t	P
1	1.991	NS	3.153	<0.05	0.306	NS	3.790	<0.02
2	3.505	<0.02	7.446	<0.001	0.600	NS	3.939	<0.02
3	2.824	<0.05	4.398	<0.01	1.318	NS	3.064	<0.05
4	3.245	<0.05	3.508	<0.02	1.381	NS	4.203	<0.01
5	2.540	NS	2.786	<0.05	1.508	NS	3.414	<0.02

[a] Paired t comparison using each animal as its own control. $n = 6$. [b] NS indicates $P > 0.05$ based on 5 degrees of freedom.

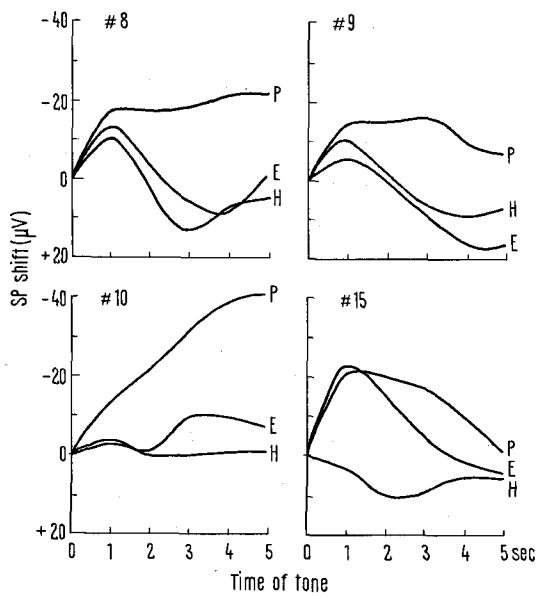

Fig. 2. Averaged steady potential shifts recorded from individual animals during 80 habituation trials (H), 80 pairing trials (P) and 80 extinction trials (E).

and COHEN[7] and CHIORINI[6] regarding the types of SP response changes which occur during habituation, conditioning and extinction.

The results with this experimental procedure suggest that the SP response to tone during pairing with foot-shock is related to the animal's anticipation or expectancy of the impending shock. This idea is further supported by the fact that the SP response was unchanged during the early extinction trials but then gradually diminished as the animals 'learned' that the shock was no longer imminent. Previous studies have shown a relationship between the SP response associated with lever-pressing for a food reward and the 'anticipation' of the number of rewards that would be received[15].

In the present experiments the SP response during pairing could also be considered a correlate of the conditioned emotional response. This would suggest that it may be worthwhile to study the effects of psychoactive drugs on such SP responses. The possible similarity between these SP responses to warning stimuli and the human CNV, which is known to be influenced by psychological state[16], further indicates the importance of a thorough examination of SP-behavior relationships.

In conclusion, this experimental procedure provides reproducible SP response patterns, similar to those observed by other investigators, which can be utilized in an investigation of the role of brain steady potential changes in the action of psychoactive drugs.

Zusammenfassung. Während Versuchsreihen an Ratten über Gewöhnung, Paarung und Auslöschung wurden jeweils signifikante Veränderungen des kortikalen Bestandespotentials (Gleichspannung) als Antwort auf Schallreiz gemessen.

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Inhibitory Effect of Bile Acids on Renin Angiotensinogen Reaction System

We have already reported that the naturally occurring renin inhibitor in ox bile was taurodeoyxcholic acid[1] and that synthetic sodium deoxycholate was also a potent competitive inhibitor of renin[2]. In this investigation, we examined the effect of various synthetic bile acid derivatives on the activity of renin in the formation of angiotensin in vitro.

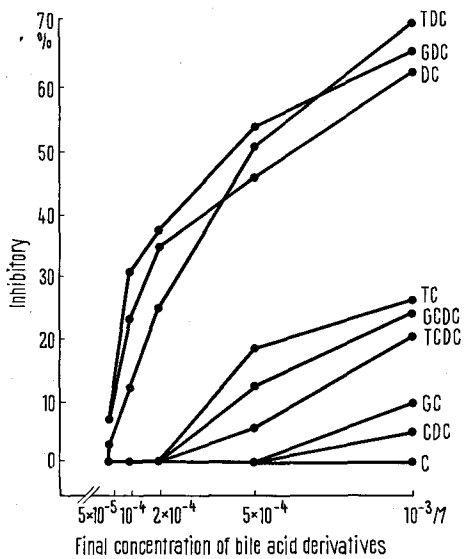
Materials and methods. Taurocholic acid, glycocholic acid, taurodeoxycholic acid, glycodeoxycholic acid, chenodeoxycholic acid, taurochenodeoxycholic acid and

glycochenodeoxycholic acid (sodium salt) were purchased from Calbiochem Co., USA. Sodium deoxycholate was obtained from Difco Laboratories, USA. Cholic acid (sodium salt) was supplied from the Department of Biochemistry, Hiroshima University, and synthetic Val^5-angiotensin II amide from the Ciba Pharmaceutical Institute.

Renin was prepared from rabbit renal cortex using the method of HAAS et al.[3] followed by ammonium sulfate fractionation between 30 to 60% saturation and dialysis against physiologic saline containing $2 \times 10^{-3} M$ EDTA. The activity of renin was equivalent to 17.3 μg of Val^5-angiotensin II amide per ml of the preparation (protein amount 10.8 mg/ml), according to the direct method[4]. The renin preparation was diluted 20-fold with physiologic saline prior to use.

Renin substrate was prepared from the heparinized plasma of rabbits nephrectomized bilaterally, according to the method of SEN et al.[4], 24 h before use. This preparation contained renin substrate equivalent to 18.0 μg of angiotensin per ml (protein amount 9.3 mg/ml), according to the indirect method of PICKENS et al.[5]. The substrate used for experiments was diluted 5-fold with physiologic saline.

The reaction mixture consisted of 0.2 ml of renin preparation, 0.5 ml of renin substrate, 0.1 ml of distilled water containing a test material and 1.3 ml of $1/15 M$ phosphate buffer, pH 6.4. The mixture was incubated at 37 °C for 10 min and the reaction was stopped by heating in a boiling water-bath for 5 min. The control assay system without a test material produced usually 0.15–0.16 μg/ml of angiotensin. The renin reninsubstrate reaction in the assay condition was a first order reaction[1]. The angiotensin formed was assayed by means of its pressor response in rats as described before[1,2]. The inhibitory effect was expressed as a percentage reduction in the angiotensin formation of the control assay system, that is, inhibitory percent.



Inhibitory effects in the presence of various amounts of bile acid derivatives on the angiotensin formation of renin. C, cholic acid; TC, taurocholic acid; GC, glycocholic acid; DC, deoxycholic acid; TDC, taurodeoxycholic acid; GDC, glycodeoxycholic acid; CDC, chenodeoxycholic acid; TCDC, taurochenodeoxycholic acid; GCDC, glycochenodeoxycholic acid. Percent of inhibitory see text.

Results and discussion. The Figure shows the inhibitory effects in the presence of various concentration of bile acid derivatives (final concentration: 5×10^{-5}, 10^{-4}, 2×10^{-4}, 5×10^{-4} and $10^{-3} M$) on the angiotensin formation of renin.

Only deoxycholic acid, taurodeoxycholic acid and glycodeoxycholic acid among the synthetic compounds examined had a potent inhibiting effect on angiotensin formation of renin. The inhibitory percent increased as the amount of these bile acid derivatives added to the assay system increased. About 50% reduction in angiotensin formation was found at a concentration of $5 \times 10^{-4} M$ of the derivatives in the assay system.

An inhibition of angiotensin formation of renin with the other bile acid derivatives, cholic acid, taurocholic acid, glycocholic acid, chenodeoxycholic acid, taurochenodeoxycholic acid and glycochenodeoxycholic acid, could not be proved till the concentration $2 \times 10^{-4} M$, and it was markedly smaller in higher concentration than in the 7-deoxy bile acids.

The facts suggest that the 7-deoxy form of bile acids might be essential for renin inhibition. The following phenomena have been confirmed in the previous report[2], that is, 1. sodium deoxycholate neither modified the physiological pressor activity of angiotensin, nor showed hypotensive effect, 2. it did not inactivate renin substrate under the conditions of the experiment, 3. the preincubation of renin with sodium deoxycholate resulted in no reduction of enzyme activity in vivo (pressor activity) and in vitro (angiotensin formation). It was also demonstrated that the inhibitory effects of these compounds were competitive according to the Lineweaver-Burk-type plot.

Zusammenfassung. Die Wirkung verschiedener synthetischer Derivate der Gallensäure wurden auf die Reninaktivität in Form des Angiotensin in vitro untersucht und es wird darauf hingewiesen, dass die 7-Desoxyform der Gallensäuren für die Renininhibition wesentlich ist.

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[6] The authors wish to thank Dr. T. HOSHITA of Department of Biochemistry, Hiroshima University, for his kind supply of cholic acid.