Some Observations on the Mechanism of Action of Baclofen (β-Chlorophenyl-γ-Amino-Butyric Acid)

A considerable amount of evidence suggests a transmitter role for γ-amino-butyric acid (GABA) as an inhibitory transmitter in the mammalian brain[1]. Fewer experimental data supporting the hypothesis that GABA functions as a neurotransmitter in the mammalian spinal cord exist, but it may play a role in presynaptic inhibitory mechanisms[2]. Involvement of GABA in spinal inhibitory systems would be of considerable interest in a pharmacological approach to spinal spasticity where an enhanced activity of excitatory pathways may be responsible for the symptomatology[3]. Since GABA does not penetrate the blood-brain barrier[4], a derivative which can cross this barrier, baclofen (β-chlorophenyl-γ-amino-butyric acid, 'Lioresal') has been used in the treatment of spastic patients with beneficial results[5]. Whether the therapeutic value of baclofen is attributable to a GABA-like action has recently been questioned on the basis of electrophysiological experiments on the cat brain and spinal cord[6,7]. Because of the intrinsic complexity of the mammalian CNS, we decided to examine the effect of baclofen on a less complex synaptic region, the lobster neuromuscular junction where GABA is recognized as being the inhibitory neurotransmitter[8]. Moreover, as an antispastic agent would be expected to depress the motor neurone activity, the action of baclofen on spinal acetylcholine (ACh) output, which mostly originates from motor neurone fibres[9], was also investigated.

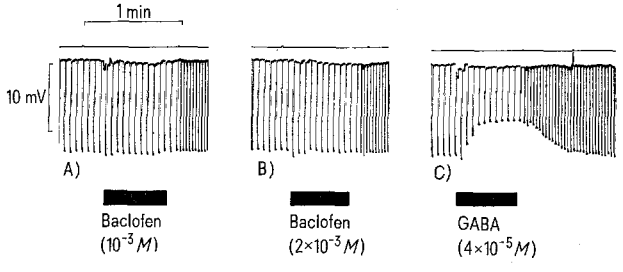

Fig. 1. Hyperpolarizing electrotonic potentials produced by rectangular inward current pulses (4×10^{-7} A; 800 msec; 0.25 Hz) recorded at the middle of a single lobster muscle fibre (within 50 μm of a central current microelectrode) in the claw opener muscle of the first walking leg (membrane potential −76 mv). The muscle was bathed with drug solution during the periods indicated by the bars below each tracing. A) and B) show effects of 10^{-3} M and 2×10^{-3} M Baclofen respectively. C) shows effect of 4×10^{-5} M GABA. The reduction in amplitude of the hyperpolarizing potentials during application of GABA, indicates an increase in membrane conductance. Normal chart speed was 50 mm/min. Note slower chart speed (25 mm/min) during offset of responses.

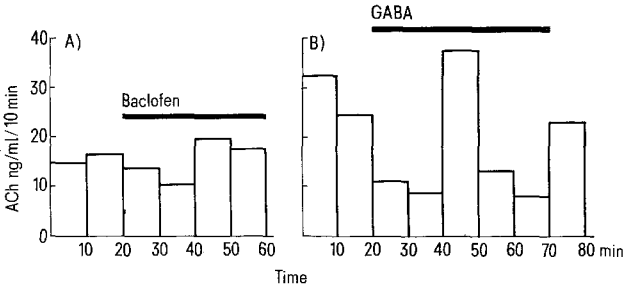

Fig. 2. Two representative experiments showing the effect of Baclofen (2×10^{-4} M) (A) and GABA (10^{-4} M) (B) on the spontaneous ACh release from the frog spinal cord. Abscissa: time (minutes). Ordinate: ACh release expressed as ng of ACh per ml of incubation medium in consecutive 10 min periods.

Methods. Fibres in the claw-opener muscle of the walking leg of the lobster (*Homarus vulgaris*) were exposed and perfused continuously with a crustacean Ringer solution[10]. Membrane conductance was measured by the method of TAKEUCHI and TAKEUCHI[11]. The membrane potential at the centre and tendon end of a single superficial muscle fibre was monitored continuously, using 2 intracellular glass microelectrodes (tip diameter < 1 μm) filled with 2 M K-citrate or 3 M KCl. A 3rd microelectrode filled with 0.6 M K_2SO_4 was also inserted at the centre of the fibre within 50 μm of the voltage electrode and was used to pass constant hyperpolarizing current pulses (800 msec; 0.25 Hz) through the membrane. The resultant electronic potentials were monitored on a Tektronix 502A oscilloscope and recorded on a Devices MX4 chart recorded. All the experiments were carried out at room temperature.

The spontaneous ACh output from the frog spinal cord was studied by the method of MITCHELL and PHILLIS[9]. Frogs (*Rana temporaria*) were decerebrated and the spinal cord removed, hemisected and incubated in a 500 μl bath at 14 °C. Every 10 min, the incubation fluid[9] was collected and bioassayed on the dorsal muscle of the leech[12].

Results. The membrane conductance of the lobster muscle fibre was not significantly altered by the application of baclofen (10^{-4} to 10^{-2} M) (Figure 1, A and B). Conversely, the administration of GABA (10^{-5} to 10^{-3} M) was followed by large increases in membrane conductance (Figure 1, C) as previously reported[13,14]. Baclofen had no effect on the GABA dose/conductance curve.

In 4 experiments, the spontaneous ACh output from the frog spinal cord was reduced by the application of baclofen (2×10^{-4} M). A 35.0 ± 9.0% (mean ± SEM) decrease in ACh output was observed over a period of 20 min, after which the ACh efflux returned to the pretreatment level, despite the continued presence of baclofen. An example of this effect is shown in Figure 2, A. The reduction in ACh release produced by GABA (10^{-4} M) in the first 10 min of application was more marked than that produced by baclofen, and over a period of 20 min it showed an average value of 48.0 ± 7.5% (Figure 2, B). This effect of GABA lasted as long as this substance was administered, although a transient return of ACh

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output to the control values was often observed (Figure 2, B). Inactivation or transport processes of these 2 substances might account for the observed recovery in the ACh values.

Discussion. GABA is the inhibitory transmitter at the crustacean neuromuscular junction[8] where it increases membrane conductance to Cl^-[11], and it is suggested as a mediator of presynaptic inhibition in the amphibian spinal cord[15,16]; therefore in the latter a reduction in ACh output after the administration of GABA may be the result of increased inhibitory influences on motor neurones which are the main source of the ACh released from this preparation[9]. Baclofen (10^{-4} to 10^{-2} *M*) did not act in a similar way to GABA at the lobster neuromuscular junction, and at the dose of 2×10^{-4} *M* had a weaker and shorter action than GABA (10^{-4} *M*) in the frog spinal cord. This accords with previous findings on the cat CNS[6,7] where baclofen is less potent than GABA and seems to have a different mechanism of action. In the spinal cord, however, the administration of baclofen depresses the neuronal firing[6] and the ACh release; two findings which might account for its antispastic action. CURTIS et al.[6] and DAVIES and WATKINS[7] suggested that the effect of baclofen might derive from an interaction with catecholamine receptors. It seems therefore that baclofen does not act via GABA mechanisms. The precise mechanism of action of this drug remains yet to be elucidated.

Riassunto. Il Baclofen (acido β-clorofenil-γ-aminobutirrico), a differenza del GABA, non modifica la conduttanza di membrana della fibra muscolare di aragosta. Il Baclofen riduce la liberazione di acetilcolina dal midollo spinale di rana; tale effetto è minore di quello prodotto dal GABA. Le due sostanze sembrano agire attraverso differenti meccanismi.

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Renal Effects of the Prostaglandins A_1 and E_2 in Hydrated and Hydropenic Dogs

Differences between prostaglandin A_1 and E_2 (PGA_1, PGE_2), with respect to their effects on renal blood flow and fluid and electrolyte excretion by the dog kidney, have been reported recently[1-3]. PGA_1 increases renal blood flow in maximally effective doses by approximately $^1/_3$ with only weak effects on excretory parameters, whereas PGE_2 increases blood flow by up to 80% and urine output by more than 400%. Since these experiments were performed in dogs with their fluid metabolism in steady state undergoing a slight NaCl-diuresis, no evidence could by obtained on the effects of the prostaglandins on the renal handling of free water. On the other hand, effects on free water excretion can be expected, since it is well known that prostaglandins can stimulate the formation of cAMP and that PGE_2 inhibits the effects of ADH in isolated collecting ducts[4]. In the study reported here, we infused PGA_1 and PGE_2 at the maximally effective rate[2] of 1 µg/min into 1 renal artery of dogs which were either in hydropenic state or undergoing water diuresis to evaluate the effects on free water reabsorption and excretion. In addition, the urinary excretion of cAMP was determined in the antidiuresis experiments.

Methods. The experiments were performed in 5 mongrel dogs of either sex weighing 17–20 kg. 3 'hydropenic' dogs were deprived of water 36 h prior to the experiments with free access to dry food. They received 20 U vasopressin after the induction of anesthesia, and an infusion at 3.5 ml/min during the surgical procedure and at 1.5 ml/min throughout the experiment which contained 147 mEq/l Na^+, 4 mEq/l K^+, 140 mEq/l Cl^- and 11 mEq/l HCO_3^-. The experiment was not begun unless urinary osmolality was at least 1000 mosmol/l. 2 'hydrated' dogs were deprived of food 24 h prior to the experiments with free access to water. They received an infusion of 0.3 *M* glucose at 10–12 ml/min after induction of anesthesia for 90 min during the surgical procedures and an infusion of 0.15 *M* glucose, 73.5 mEq/l Na^+, 2 mEq/l K^+, 70 mEq/l Cl^- and 5.5 mEq/l HCO_3^- at 10 ml/min throughout the experiment. The experiment was not begun unless urinary sodium concentration was below 15 mEq/l.

Anesthesia, surgical procedure, infusion of prostaglandins[5] into the left renal artery, general protocol of the experiments, and determination of renal blood flow with an electromagnetic flow meter, of glomerular filtration rate (GFR) as clearance of creatinine and of the excretion of fluid and electrolytes were carried out as described previously[1,2]. The concentration of cAMP in the urine of the infused kidney was determined by a radio isotope dilution test using the test kit of Boehringer/Mannheim. To discriminate effects by the prostaglandins from changes of other variables, all parameters from the experimental kidney except blood flow and C_{H_2O} or Tc_{H_2O}, were related to the control kidney. The ratios of experimental (left) kidney/control (right) kidney during the infusion of one of the prostaglandins were expressed in percent of the ratios during the corresponding control periods.

Results and discussion. None of the 2 prostaglandins A_1 and E_2 affected systemic blood pressure or GFR at the doses applied here. The effects of the 2 prostaglandins on blood flow (BF), urinary flow rate (UV) and osmolar clearance (C_{osm}) are demonstrated in the Figure. PGA_1

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