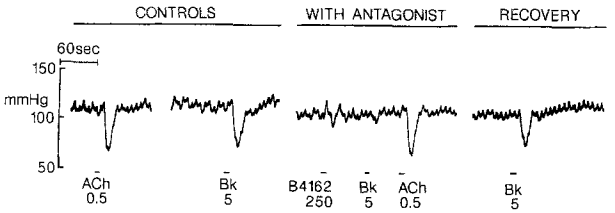

Figure 3. Cat 4.3 kg ♀. Hypotensive effect of acetylcholine (ACh, 0.5 nmole i.v.) and bradykinin (Bk, 5 nmole i.v.) before and immediately after injection of Bk antagonist (B4162, 250 nmole, i.v.). Partial recovery occurred 5.5 min after injection of the antagonist.

values, respectively. The results of these seven experiments are shown in figure 2. It shows that the Bk antagonist B4162 (50 nmole i.a.) greatly reduced the vasodilator response to Bk (0.1 nmole i.a.) but had no effect on chorda-lingual nerve stimulation (5 s). The same result was obtained if the nerve was stimulated for 1, 2 or 10 s. (not shown in the figure). The vasodilator responses to ACh injected close-arterially and to sympathetic nerve stimulation (an 'after' dilatation) were also unaffected by the Bk antagonist.

Effect of bradykinin antagonist (B4162) on hypotensive responses to bradykinin. Bk (1–100 nmole i.v.) caused a fall in arterial blood pressure in cats (n = 4) and rabbits (n = 3). The hypotensive response was reduced in a dose-dependent manner by the Bk antagonist. The molar ratio of antagonist to agonist resulting in 50% reduction of the Bk response was 2.4, 6 and 9 in three experiments in cats, and 1.8 and 3 in two experiments in rabbits. The antagonism was temporary, with 50% recovery from complete inhibition in 2–6 min. Similar results have also been obtained in guinea-pigs (Barton, Padsha and Schachter, unpublished). Figure 3 shows a typical result of an experiment in a cat in which the Bk antagonist B4162 (250 nmole i.v.) abolished the hypotensive response to Bk (5 nmole i.v.) but had no effect on that to ACh (0.5 nmole i.v.). The specificity of B4162 was further demonstrated in other experiments (2 cats, 1 rabbit) in which it was shown to have no effect on the hypotensive responses to VIP (1–5 nmole i.v.) and substance P (5 nmole i.v.).

Discussion. Our results show that it is possible to abolish the vasodilatation produced by bradykinin injected close-arterially into the submandibular gland of the cat whilst that produced by nerve stimulation is unaffected. This confirms the view that the kallikrein-kinin system does *not* mediate functional hyperaemia in the cat submandibular gland [8, 9]. Studies carried out on the submandibular gland of the rat, which has one of the highest concentrations of tissue kallikrein in mammalian organs, led to the conclusion that in this species salivary kallikrein mediates the nerve-induced vasodilatation [10]. In a recent report these authors found that the intra-arterial infusion of the same Bk antagonist used in our studies, blocked the vasodilator response of the rat submandibular gland to Bk [11]. The vascular response to nerve stimulation, however, was not tested in the latter experiments. Further studies are still needed to clarify the significance of salivary kallikrein and of the kinin which it can release.

These new Bk antagonists are likely to prove most valuable. The synthesis of analogues with longer duration of action would make them even more useful for investigating the significance of Bk in physiological and pathophysiological conditions. There is also reason to expect that they will have some therapeutic applications in humans.

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Corticosteroid effects on cholinergic enzymes in ethanol-treated fetal brain cell cultures

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Summary. In the presence of ethanol, corticosterone and dexamethasone inhibit choline acetyltransferase and acetylcholinesterase activities in cultured fetal brain cells of the rat. These results suggest that corticosteroids may have an important influence on the activity of cholinergic enzymes in the fetal brain and may antagonize the effects of ethanol in this setting.

Key words. Fetal brain cells; ethanol; corticosterone; dexamethasone; choline acetyltransferase; acetylcholinesterase.

Recent studies have demonstrated stimulatory effects of ethanol (ETOH) on cholinergic enzymes in brain cells of the fetal rat [1]. Combined in utero and in vitro ethanol exposure has been found to result in elevations of both choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities. These effects could contribute to the cognitive and neu-

rologic dysfunctions (mental retardation, hyperactivity, hypotonia, and microcephaly) observed in the 'fetal alcohol syndrome'[2-4]. Since it has been shown recently[5] that both naturally-occurring and synthetic glucocorticoid hormones have the capacity to inhibit ChAT and AChE in neuronally-transdifferentiating adrenal medullary cells, a study was undertaken to determine whether these compounds could prevent the stimulatory effects of ETOH on fetal brain ChAT and AChE in the rat.

Methods. Sprague-Dawley rats were obtained from the Charles River Breeding Laboratories (Wilmington, MA) on their 12th day of pregnancy and housed individually in clear plastic cages. Animals were fed Purina Rat Chow and water ad libitum and maintained on a 14:10 h light:dark cycle, with a controlled temperature of 23 ± 1 °C. Twenty-two days after fertilization, mothers were anesthetized with CO_2 and the fetuses delivered by Caesarean section. Fetuses were then sacrificed and brains removed and transferred into an enzyme solution containing papain (0.1%, w/v) and DNAase (15 µg/ml) in sterile phosphate-buffered saline. Brain tissues from approximately 8–10 fetuses were pooled; after preliminary mechanical dissociation in this solution, tissue was warmed to 37 °C and further digested by shaking for 20 min. Free cells were aspirated and centrifuged (3000 rpm for 40 s). The resulting pellet was dispersed in Medium 199 and recentrifuged. The final pellet was suspended in Medium 199 supplemented with 15% (v/v) fetal calf serum and standard GIBCO 1 × antibiotic/antimycotic mix. Corticosterone or dexamethasone, each 10^{-5} M, was added in ETOH (0.1%, v/v) to separate cultures. Other incubations were carried out with no additives (control) or with ETOH alone. Cells were maintained in culture with these agents under a 95% air/5% CO_2 gas phase for 5 days. After this incubation, cultures were washed extensively and cells frozen and later assayed for ChAT and AChE by the spectrophotometric methods of Chao and Wolfgram[6] and of Ellman et al.[7], respectively; neither ETOH nor hormones were present during actual enzyme determinations.

Results and discussion. Figure 1 shows a photomicrograph of cultured brain cells from 22-day-old fetal rats. This mixed cell-type whole brain primary culture contains not only neurons but also astrocytes, oligodendrocytes, and endothelial cells. Cholinergic neurons comprise a significant although undetermined fraction of cells in these cultures, however, since both ChAT (an enzyme specific for cholinergic neurons) and AChE (an enzyme found in cholinergic neurons and glial elements, as well as other cell types) were present in significant activities. In other studies[8,9] using animals of comparable ages, catecholamines and various other neuroectoderm-specific substances (e.g., luteinizing hormone releasing hormone) have been demonstrated histochemically in similarly prepared primary brain cell cultures.

The presence of ethanol, either with or without corticosteroids, did not affect the survival or light microscopic appearance of cells in these cultures. In other studies[5], we have shown that changes in the morphology of adrenal chromaffin cells do occur when they are exposed to corticosteroids in similar culture systems for periods longer than 5 days.

Figure 2 depicts ChAT activities in brain cells from 22-day-old fetuses under various experimental conditions. ETOH exposure in vitro (0.1%, v/v) had a significant stimulatory effect on this enzyme (453% of control activity; $p < 0.005$). The presence at 10^{-5} M concentration of either corticosterone or dexamethasone abolished this ETOH stimulation and reduced levels of ChAT activity to or below unstimulated values (51 and 92% of control for corticosterone and dexamethasone, respectively).

AChE activity (fig. 3) was not significantly stimulated by in vitro ETOH exposure alone. Other studies[1] indicate, however, that combined in utero and in vitro exposures to ETOH

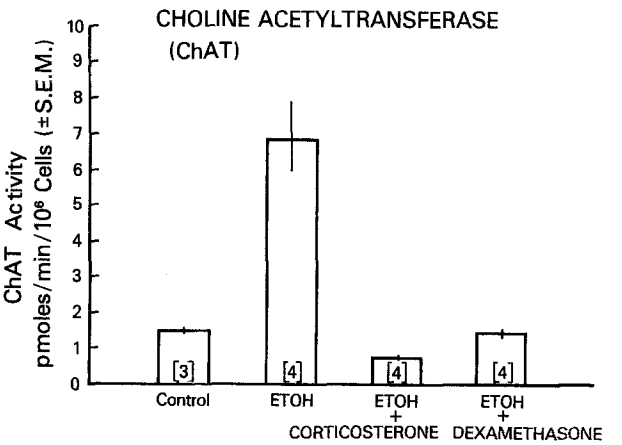

Figure 2. Choline acetyltransferase (ChAT) activity in cultured brain cells from 22-day-old fetal rats. Activities are shown in control incubations; in cultures with 0.1% (v/v) ethanol (ETOH); with 10^{-5} M corticosterone and ETOH; or 10^{-5} M dexamethasone and ETOH. Values indicated are the means ± SE of [n] determinations.

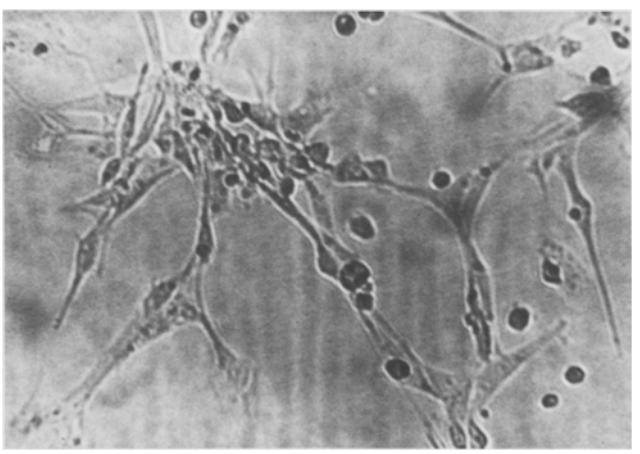

Figure 1. Primary cell cultures derived from whole brain tissue obtained from 22-day-old fetal rats, magnification ×400.

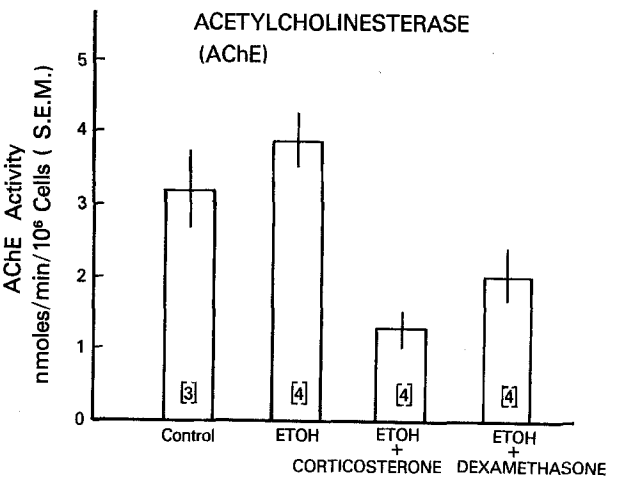

Figure 3. Acetylcholinesterase (AChE) activity in cultured brain cells from 22-day-old fetal rats. Activities are shown under treatment conditions identical to those indicated for figure 2.

can indeed result in marked increases in the activity of this enzyme. In the investigation described here, both corticosterone and dexamethasone strongly depressed AChE activity in the presence of ETOH (41 and 65% of control; $p < 0.005$ and 0.05, respectively).

These studies thus demonstrate that corticosteroid hormones can abolish the ETOH-induced stimulation of ChAT (a rate-limiting enzyme of acetylcholine synthesis) in cultured fetal brain cells of the rat and can further reduce enzyme activity to below unstimulated values. In the presence of ETOH, corticosteroids were also shown to decrease the measured activity of AChE, a key enzyme involved in acetylcholine degradation. More extensive studies will be required to determine the mechanism of action for these steroid hormones in this system and also to determine whether such compounds may have a protective influence on neurologic and behavioral development during fetal alcohol exposure.

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Testosterone changes the electric organ discharge and external morphology of the mormyrid fish, *Gnathonemus petersii* (Mormyriformes)[1]

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Summary. Effects of silastic and pellet methyltestosterone implants on the waveform of the electric organ discharge of the weakly electric African mormyrid, *Gnathonemus petersii*, were investigated. Within seven days of implantation, the duration of the discharge increased dramatically while the associated peak power frequency of the Fourier spectrum decreased in all treated fish. By day 35, hormone-treated fish exhibited up to five-fold increases in EOD duration, as well as multiple discharges and variations in the shape of the positive phase of the discharge. Testosterone treatment also changed body morphology, making immature and adult female fish resemble adult males.
Key words. Gnathonemus petersii; Mormyridae; electric organ discharge; testosterone; sex differences.

Several species of mormyrid fish exhibit sex differences in the characteristics of their electric organ discharge. Such differences appear important for sexual identification[3]. However, the majority of mormyrids investigated failed to exhibit any sexual dimorphism in waveform or discharge pattern under laboratory conditions[4]. Male hormones administered to juvenile or adult females typically result in fish exhibiting male-like discharges[5]. However, only when a natural sex difference is evident under laboratory or field conditions, is it affected by exogenous gonadal hormone manipulation[5]. Thus, it is believed that treatment with gonadal steroids may predict naturally occurring sexually dimorphic electric-organ-discharge (EOD) waveforms[5].

Kramer and Westby[6] did not find a sex difference in the waveform of the EOD of *Gnathonemus petersii*. Landsman, Jou and Moller[7], however, reported a sex difference in the average peak power spectral frequency (PPSF) for this species. Only when the fish were recorded at rest, unrestrained in their shelters, did males exhibit peak power spectra of higher frequencies than females, with some overlap between the sexes. This sex difference was inversely related to the duration of the EOD, with male EODs shorter than those of females.

The present study investigated whether the EOD waveform of *G. petersii* can be influenced by testosterone, to substantiate a natural EOD sex difference in this species.

Materials and methods. Animals. Fourteen juvenile *G. petersii* (standard length: 10.2–12.0 cm; weight: 10.0–19.0 g) and one adult female (16.1 cm, 42 g) were randomly selected from large stock tanks (Lombardos African Fish Imports, Newark, N. J.). Fish were maintained individually in 20-l aquaria on a 12:12 L:D cycle, with lights on at 09.00 h. Water conductivity was kept at 200 ± 40 µS/cm and temperature at 23 ± 0.5 °C. *EOD recordings.* A pair of Ag/AgCl electrodes, extending from plexiglas tubes were fitted to the far ends of the aquarium, approximately 8 cm from head and tail of the fish when at rest in its porous ceramic shelter. EODs were fed directly into an oscilloscope (Tektronix, model 455) which triggered a spectrum analyzer (Hewlett-Packard, model 3582A, range: 0–25 KHz, resolution: 100 Hz). Both EODs and Fourier transformations were plotted with an X-Y plotter (Hewlett-Packard) and photographed from the screens of the oscilloscope and spectrum analyzer, respectively. All recordings were made between 15.00 h and 17.00 h.

Gonadal manipulation and hormone implants. The duration of the EOD was tested in each fish. Then, three of the juveniles were anesthetized (tricaine methane sulfonate, 1:20 000) and either 1) implanted with a silastic capsule (Dow Corning 0.065 inch o.d. and 0.03 inch i.d.) containing 1 mm/2.5 g b.wt packed 17α-methyltestosterone (17α-T) (Sigma), 2) gonadectomized and implanted with a similarly sized 17α-T