TITLE: **PENTOBARBITAL INCREASES cAMP ACCUMULATION IN S49 CELLS INDEPENDENT OF GUANINE NUCLEOTIDE BINDING PROTEINS.**

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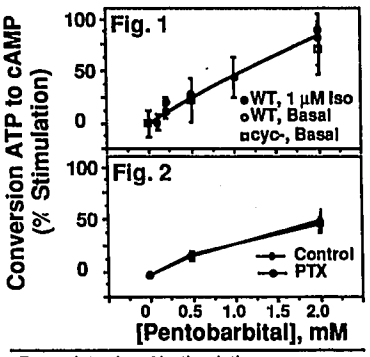
Anesthesia is augmented or produced following administration of agonists at α_2 receptors such as clonidine or dexmedetomidine[1,2]. These compounds inhibit cAMP formation by a receptor mediated mechanism dependent on a guanine nucleotide binding (G) protein. To investigate the effects on cAMP accumulation by other anesthetics, and to further study the role of G proteins, the effect of pentobarbital on cAMP accumulation in S49 cells was studied. WT S49 cells contain β-adrenergic receptors, the heterotrimeric stimulatory and inhibitory G proteins (G_s, G_i) and adenylyl cyclase. Cyc$^-$ S49 cells do not express $G_{s\alpha}$, which is the subunit of G_s that binds GTP[3].

Cyclic AMP accumulation was assayed by measuring the conversion of [^{3}H]ATP to [^{3}H]cAMP[4]. Cells were incubated with [2,8-^{3}H]adenine, which is taken up by the cells and converted to [^{3}H]ATP. The cells were washed and incubated with drugs in the presence of 0.5 mM IBMX for ten minutes prior to stopping the reaction with trichloroacetic acid. [^{3}H]cAMP and [^{3}H]ATP were separated by chromatography over dowex and alumina columns. Activity is reported as the percentage of ATP converted to cAMP. To define the role of G_i, cells were pretreated with pertussis toxin (PTX, 200 ng/ml) for 16 hours[4]. PTX catalyzes the ribosylation of G_i and interferes with its normal function.

Pentobarbital increased cAMP accumulation in S49 cells (Figure 1). The degree of stimulation by pentobarbital was the same for WT cells in the presence or absence of isoproterenol as well as for cyc$^-$ cells under basal conditions; this demonstrates that the effect is not dependent on $G_{s\alpha}$. Pretreatment with pertussis toxin abolished the ability of 1 μM somatostatin to inhibit cAMP accumulation stimulated by 6 μM forskolin (data not shown), but had no effect on the response to pentobarbital (Figure 2), suggesting that inhibition of the function of G_i is not involved in the effect of pentobarbital.



Data plotted as % stimulation over control (0 mM pentobarbital) activities for each cell type and condition.

CONTROL ACTIVITIES:		
Cell Type	Condition	% Conversion
WT:	1 μM Isoproterenol	4.4 ± 0.04
	Basal	0.29 ± 0.01
Cyc-	Basal	0.031 ± 0 .007

(mean ± sem, N=3-8.)

References: 1. *Anesthesiol.* **69**:818, 1989.
2. *Anesthesiol.* **71**:75, 1989.
3. *Adv. Cyclic Nucleotide Res.* **13**:1, 1980.
4. *Biochem. Pharmacol.* **37**:4289, 1988.

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TITLE: EFFECT OF TRANSCUTANEOUS CRANIAL ELECTRICAL STIMULATION ON HALOTHANE REQUIREMENTS IN RATS

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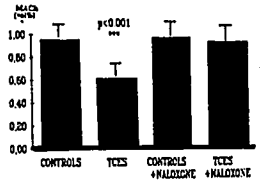
Transcutaneous cranial electrical stimulation (TCES) has been shown to increase the analgesic effect of nitrous oxide and narcotics.[1,2] In contrast, no data regarding the effects of TCES on the anesthetic requirements for halogenated anesthetics are yet available. This study was thus designed to assess the influence of TCES on halothane requirements in rats.

Eighteen male rats were implanted with 3 silver electrodes (one frontal and two posterior ones) connected to a microplug fixed to the cranium. Following a 5 days recovery period, the animals were connected to a 2 channel electric generator and randomly allocated to be either stimulated (TCES, n = 9) or not (Controls, n = 9). Electrical stimulation was begun 12 hr before the experiment and applied during the study period by using a high frequency intermittent biphasic current (100 mA intensity, 166 kHz applied for 4 ms at 100 Hz in a 2 us postive, 4 us negative sequence). Rats were mechanically ventilated with halothane in 100% oxygen and ventilation adjusted to maintain normocarbia. Rectal temperature was servocontrolled to 37°C. MAC for halothane (MACh) was determined using a standard up and down tail clamp technique. MACh was defined as the end-tidal halothane value which prevented the motor response to a 60 sec noxious tail clamp in 50 % of the animals. At the end of the experiment, rats were given a 2 mg/kg subcutaneous naloxone dose and MACh again determined. Statistical significance ($p<0.05$) was assessed using the paired and unpaired Student's t tests. Results are expressed as mean±SD.

MACh was significantly decreased in TCES animals when compared with controls ($p<0.001$, figure). After naloxone administration, MACh was restored to control values in TCES rats but was unchanged in non stimulated animals.

Figure



These results indicate that TCES decreases halothane requirements in rats and suggest that these effects are in part mediated by endogenous opioids. The clinical relevance of these data requires further investigation in humans.

References

1. Anesthesiology 57:293-297, 1982
2. Anesth Analg 61:863-866, 1982