cAMP in guinea-pig superior cervical ganglia during preganglionic nerve stimulation[1]

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Summary. Preganglionic nerve stimulation or elevated $[K^+]_o$ increase cAMP levels in isolated guinea-pig superior cervical ganglia, a ganglion lacking adrenergic inhibitory synaptic potentials. The cAMP response to K^+ and nerve stimulation is not prevented by atropine or phentolamine. The regulation of cAMP content does not involve cholinergic or adrenergic mechanism. Of polypeptides tested, only VIP (5×10^{-6} M) increases cAMP content to the extent observed with preganglionic nerve stimulation.

Roch and Kalix[2] found that elevated $[K^+]_o$ raises the cAMP content of bovine superior cervical ganglia by a mechanism that requires Ca^{++} and is resistant to block by muscarinic and adrenergic receptor antagonists. Rat superior cervical ganglia respond to K^+ in the same way and, in addition, do not give a cAMP response to K^+ when the ganglia are denervated[3]. Moreover, electrical preganglionic nerve stimulation increases ganglion cAMP by a Ca^{++}-dependent, nonadrenergic, noncholinergic process[4]. This study of cAMP content in guinea-pig superior cervical ganglia was made because of conflicting reports about the cAMP response to preganglionic nerve stimulation[5,6]. Wamsley et al.[5] found that cAMP accumulation increases in guinea-pig ganglia treated with isoproterenol but not during preganglionic stimulation at 10 Hz for 8 min. The latter finding is consistent with the reported absence of inhibitory synaptic potentials in the ganglia[7,8]. On the other hand, Trevisani et al.[6] reported a 2-fold increase of cAMP in guinea-pig ganglia stimulated at 20 Hz for 10 min, a result incompatible with the view that cAMP mediates the ganglionic inhibitory potential.

Methods. Superior cervical ganglia were removed from male albino guinea-pigs, desheathed, and equilibrated for 30 min in Locke's solution containing 136 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.2 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 20 mM $NaHCO_3$, 5.5 mM glucose, 5–10 mM theophylline, and bubbled with 95% O_2–5% CO_2 gas. After equilibration, ganglia were transferred to solutions containing drugs to be tested. Preganglionic nerve stimulation was performed with supramaximal electrical shocks applied for 90 sec at a rate of 10 Hz. K^+ was raised to 60 mM by an equimolar decrease in Na^+. When a blocking drug was used, the ganglia were incubated in solutions containing the blocking drug for at least 10 min before the test (agonist or electrical stimulation) was applied. Ganglia were then homogenized in 6% trichloroacetic acid and the homogenate separated into supernatant and particulate fractions by centrifugation. After extraction with water-saturated ether, the supernatant solution was used for the radioimmunoassay of acetylated cyclic AMP according to the method of Steiner et al.[9].

Results and discussion. The cAMP content of resting, isolated ganglia treated with theophylline (5×10^{-3} M) is 1.9 ± 0.13/mg wet wt (table). Like superior cervical ganglia from other mammals[3], raising $[K^+]_o$ to 60 mM causes a 5.6-fold increase in cAMP content. The response to K^+ requires Ca^{++} and is not prevented by atropine (10^{-5} M; table). Similarly, preganglionic nerve stimulation at 10 Hz for 90 sec increases cAMP levels about 3-fold by a Ca^+ dependent process (table) that is not prevented by atropine (10^{-5} M) or phentolamine (10^{-5} M) applied for 40 min before and during preganglionic nerve stimulation. Like guinea-pig ganglia, but unlike rabbit ganglia[10], rat superior cervical ganglia[4] respond to preganglionic nerve stimulation with an increase in cAMP accumulation resistant block by muscarinic or adrenergic receptor antagonists. Bethanechol (10^{-4} M) has no effect on ganglion cAMP levels (table), a result consistent with the failure of atropine to alter the nucleotide response to preganglionic nerve stimulation. It should be noted that Trevisani et al.[6] found a partial, but significant, reduction in the cAMP response to stimulation of ganglia treated with atropine or phentolamine. There is no obvious explanation for this discrepancy.

It has been suggested that K^+ and nerve stimulation may cause cAMP accumulation in preganglionic axonal endings[4,6]. If this is so, then cAMP might be related to the phosphorylation of presynaptic protein I[11]. Alternatively, adenylate cyclase activation and cAMP accumulation may occur at postjunctional sites in response to unidentified substances released by K^+ or nerve stimulation[4]. It is of interest that the satellite cells in rat superior cervical ganglia contain cAMP immunoreactivity that is augmented by preganglionic nerve stimulation[12].Vasoactive intestinal polypeptide (VIP), a 28 amino acid polypeptide, causes a marked accumulation of cAMP in rat[4] and guinea-pig (table) superior cervical ganglia. VIP is found in some autonomic ganglia where its presence is suggestive of a transmitter or modulator role[13], but only a small amount of VIP-like activity is present in rat superior cervical ganglia[14]. The cAMP response of rat superior cervical ganglia to VIP does not require nerve terminals or extracellular Ca^{++}[3].

The polypeptides somatostatin (10^{-5} M) and secretin (10^{-5} M) have no effect on ganglion cAMP levels and somatostatin does not alter the effects of VIP (5×10^{-6} M) on cAMP accumulation in guinea-pig ganglia. Substance P, luteinizing hormone releasing factor and met-enkephalin do not alter cAMP levels in rat ganglia, but were not tested on guinea-pig superior cervical ganglia.

That cAMP accumulation increases during preganglionic nerve stimulation of a ganglion that does not display

Elevated cAMP levels in guinea-pig superior cervical ganglia treated with 60 mM K^+ or preganglionic nerve stimulation

	Resting cAMP content (pmoles/mg)	K^+ (60 mM) Percent of control	Electrical stimulation (10 Hz/90 sec)
Control	1.9 ± 0.13 (35)	563 ± 55 (12)	305 ± 37 (21)
Low Ca^{++}	1.3 ± 0.14 (6)	161 ± 32 (3)	131 ± 20 (6)
Atropine (10^{-5} M)	1.9 ; 2.4 (2)	474 ± 71 (6)	347 ± 35 (3)
Phentolamine (10^{-5} M)	1.6 ± 0.36 (5)	463 ± 14 (4)	260 ± 23 (4)
Bethanechol (10^{-4} M)	2.0 ± 0.18 (6)	–	–
VIP (10^{-6} M)	2.7 ± 0.02 (3)	–	–
(5×10^{-6} M)	5.3 ± 0.41 (12)	–	–

cAMP content is expressed as pmoles/mg (wet wt) and as percent of values obtained for nonstimulated ganglia. Mean values ± SE are given for the number of ganglia shown in parenthesis. For resting ganglia, only the values obtained with VIP are significantly different from control ($p < 0.01$). For stimulated ganglia, the values obtained with low Ca^{++} (0 Ca^{++}; 5 mM Mg^{++}) are different when compared with control ($p < 0.01$); the values with atropine are N.S.; the values with phentolamine are N.S. for elevated K^+ and $p < 0.05$ for electrical stimulation.

inhibitory synaptic potentials and does not contain type I small intensely fluorescent cells[15] is consistent with noncholinergic, nonadrenergic regulation of ganglion cAMP metabolism[2,4]. Whether or not prostaglandins play a role remains to be determined, but they are good possibilities for causing cyclic nucleotide accumulation during ganglionic activity[6].

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Hibernation in golden hamsters (*Mesocricetus auratus*, W.) exposed to 5% CO_2[1]

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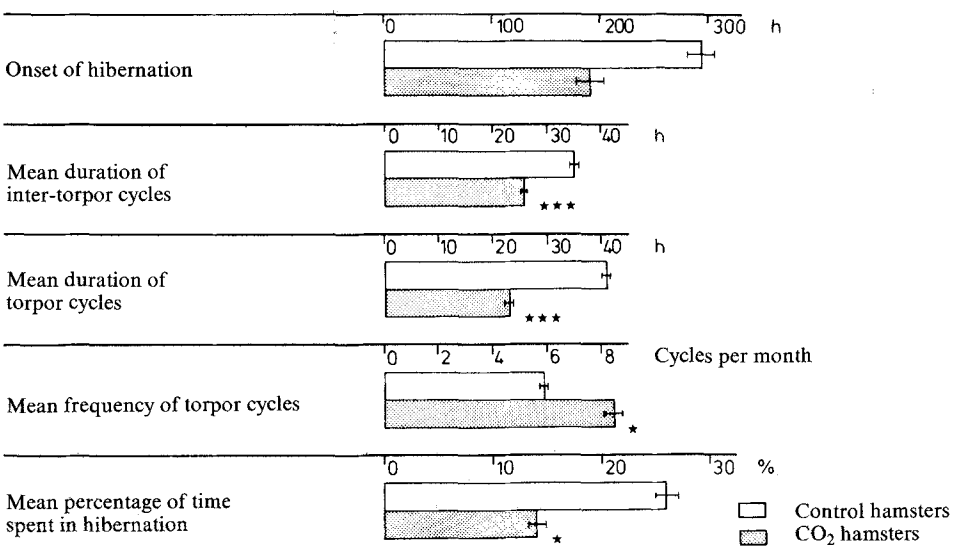
Summary. Chronic exposure of golden hamsters to a gas mixture containing 5% CO_2, 21% O_2, and 74% N_2 favors entry into hibernation. In the hibernating golden hamster, however, chronic CO_2 exposure facilitates arousal.

In the burrows of some hibernating animals the CO_2 concentration may increase considerably, as shown by Williams and Rausch[2] who measured CO_2 concentrations up to 13.5% in semiartificial dens of marmots. High concentrations of CO_2 in the inspiratory air cause a decrease of body temperatures in various nonhibernators and euthermic golden hamsters and have a direct effect on hypothalamic neurons participating in the control of body temperatures, as demonstrated in a previous study[3]. Furthermore, an increase of CO_2 concentration might affect hibernation, as postulated first by Dubois in 1896[4].

The present study in golden hamsters (Mesocricetus auratus, W.) was carried out to elucidate the effect of chronic CO_2 exposure on hibernation.

Material and methods. Experiments were carried out from December, 1981 to April, 1982 in 42 golden hamsters of both sexes weighing 81.4 ± 14.3 g (experimental animals) and 81.1 ± 18.8 g (control animals), respectively. The age of the animals was 21.0 ± 4.6 weeks. The animals were housed in individual cages at an ambient temperature of 5 ± 0.5 °C and an 8:16 h light-dark cycle. 300 ml standard hamster



The results (mean values and standard error) of 42 golden hamsters during 5 experimental months. (* $p < 0.05$; *** $p < 0.001$).