Flavonoid constituents of *Rhynchosia* species (Leguminosae)

	C-glycosides								O-glycosides			Prenylated flavonoids			
	Orientin	Isoorientin	Vitexin	Isovitexin	Vicenin-2	Vicenin-3	Schaftoside	Lucenin-2	Rutin	Kaempferol 3-rutinoside	3',4'-di-O-methyl luteolin 7-glucuronide	Tirumalin	Rhynchospermin	Apigenin	Naringenin
Rhynchosia rufescens	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–
Rhynchosia heynei	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Rhynchosia capitata	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Rhynchosia beddomei	+	+	+	+	+	–	–	+	+	–	+	–	–	+	+
Rhynchosia minima	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
Rhynchosia sericea	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Rhynchosia albiflora (R. cyanosperma)	–	–	–	–	–	–	–	–	+	+	–	+	+	–	–

–, indicates absence; +, indicates presence and isolated.

having a similar flavonoid composition. The flavonoid composition even of *R. minima,* which occupies shady habitats and of *R. beddomei,* which is predominantly adapted to dry hilly tracts, is like that of the other species. The elaboration of the flavonoids in these two species might reflect biochemical adaptation to reflect the structural elaboration of the species in different environments.

In conclusion it appears that a negative correlation exists between the leaf flavonoid profiles of *R. albiflora* and those of other species investigated. However, the situation with regard to morphological features and flavonoid content is so complex that a decision about whether this species fits into the genus *Rhynchosia* or not must await further information about other species.

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0014-4754/85/020251-02$1.50 + 0.20/0

Intracellular injection of cAMP and cGMP into snail neurones induces an increase in Na^+-conductance

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Summary. Injection of cAMP and more rarely cGMP into the neurones of the snail *Helix pomatia* induces an increase in membrane conductance, membrane depolarization and excitation. The effect is theophylline-dependent and has a reversal potential near −10mV.
Key words. Helix pomatia; snail neurones; Na^+-conductance; cAMP-injection; cGMP-injection.

Both an increase and a decrease of K^+-channel conductance induced by intracellular cAMP injection into *Aplysia* and *Helix* neurones are well documented[1–6]. However, studies of cAMP and cGMP influence on the Na^+-conductance are rather contradictory[7–9]. The present study shows that pressure intracellular injections of cAMP and more rarely cGMP into snail neurones induce membrane depolarization and a membrane conductance increase which is associated, at least partly, with an increase in Na^+-conductance.

Methods. Identified neurones of the viscero-abdominal ganglionic mass of *Helix pomatia* (cells RPa_1, RPa_2, LPa_2, and V_6 of Sakharov and Salanki[10]) were studied. The snail ganglia

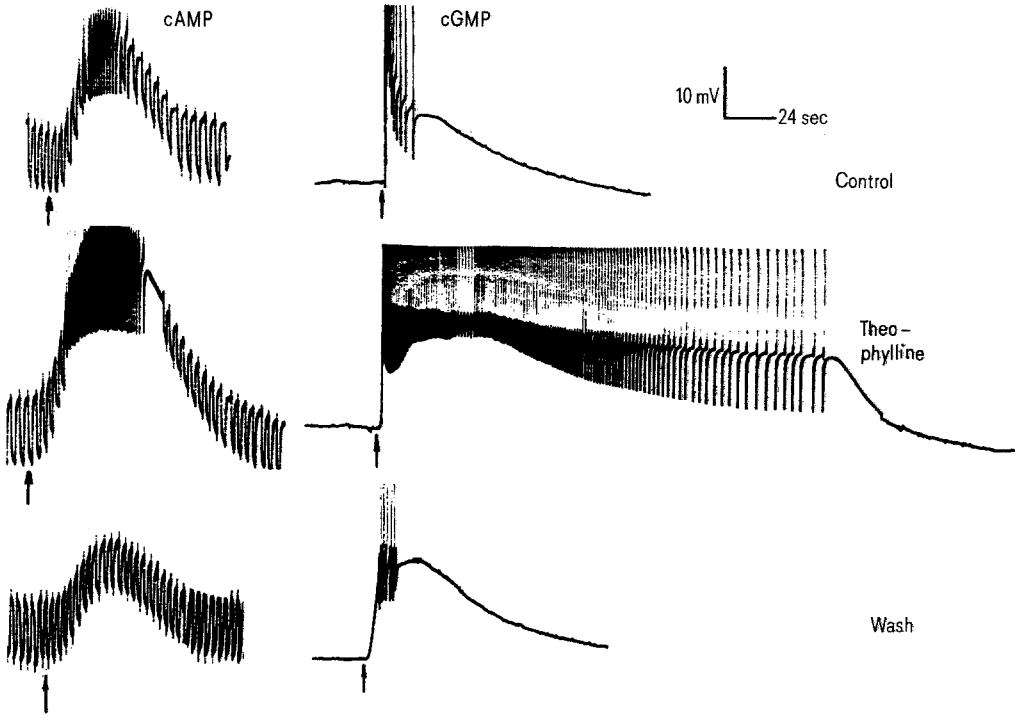

Figure 1. cAMP- and cGMP-induced depolarization of snail neurones. Left: RPa_2 cell. The membrane potential is −60 mV. The arrows indicate the moment of the cAMP injection (20 psi, 1 sec). Upper trace = the cell response on cAMP injection before theophylline injection. Medium trace = 1.5-fold increase in amplitude and duration of cAMP response 5 min following intracellular injection of theophylline. Lower trace = restoration of cAMP response to its control level 15 min after theophylline injection. Downward deflections are caused by passing outward current pulses of fixed amplitude. Membrane input resistance appeared to be reduced during the depolarizing response induced by cAMP injection. Right: V_6 cell. The membrane potential is −65 mV. cGMP injection (30 psi, 1 sec) induces depolarizing response. Upper trace = cGMP response before theophylline application. Medium trace = 2.5-fold increase in cGMP response, duration 5 min, following bath application of 0.5 mM theophylline. Lower trace = cGMP response 40 min following theophylline withdrawal.

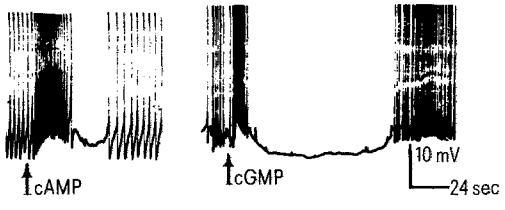

Figure 2. cAMP- and cGMP-induced biphasic responses of snail neurones. RPa_1 cell. The membrane potential is −50 mV. The arrows indicate the moment of the substances injection (30 psi, 0.5 sec).

Responses of identified snail neurones to cAMP and cGMP injection

	RPa_1 n = 8		RPa_2 m = 10		LPa_2 n = 9		V_6 n = 7	
	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
Depolarization	5	3	8	4	6	4	5	2
Biphasic responses	2	2	–	–	1	–	1	2
No effects	1	3	2	6	2	5	1	3

n, quantity of identified neurones studied in different ganglions.

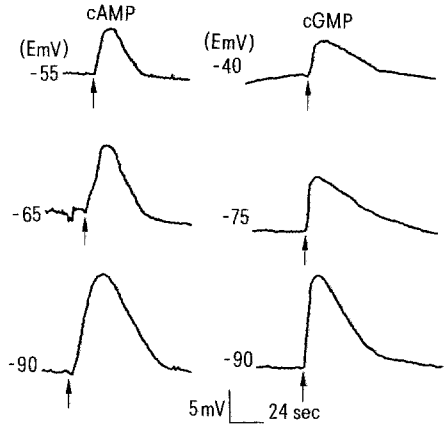

Figure 3. Dependence of the amplutide of cAMP (left) and cGMP (right) response on the membrane potential. The experiments were carried out on neurones LPa_2 of different preparations. The values of membrane potential are indicated to the left of the record. The arrows indicate the moments of the cAMP and cGMP injections.

were isolated and desheathed in a chamber and continuously bathed in 120 mM NaCl/5 mM KCl/6 mM $CaCl_2$/3.5 mM $MgCl_2$/tris-HCl, pH = 7.5–7.8. NaCl was replaced in the saline by either tris-Cl or sucrose, keeping the molarity constant. The neurones were generally impaled with a 5-barreled microelectrode. The recording barrel was filled with 2 M potassium citrate. Its resistance varied between 5 and 10 Ω. The bridge circuit was used for passing current through the recording barrel. Each of the remaining 3 barrels was filled with 0.5 M solution of cAMP (sodium salt), 0.5 M cGMP and 0.1 M theophylline (Serva). Control experiments were carried out with 0.5 M 5'AMP, 5'GMP and sodium citrate. The substances were injected by pressure 15–30 psi 0.5–2 sec. Recordings were made by the use of a high input impedance amplifier coupled to a 2-channel oscilloscope and pen and ink recorder.

Results and observations. Intracellular injections of 5'AMP 5'GMP and sodium citrate into snail neurones had no effect. The injection of cAMP and cGMP in most of the experiments elicited rapid and reversible decrease in membrane input resistance (increase in membrane conductance), evoking depolarization and excitation (fig. 1). Sometimes depolarization was

followed by a membrane hyperpolarization and inhibition of cell firing (biphasic responses, fig. 2). The latent period of responses was 0.1–2 sec and the time to peak of the first phase 0.5–25 sec. The responses of identified neurones to cAMP and cGMP injection varied in different ganglions (table).

The amplitude of depolarization became larger with membrane hyperpolarization (fig. 3). The extrapolated reversal potential of the effect was about −10 mV. Replacement of the standard Ringer solution by a Na^+-free one led in 15–20 min to the disappearance of the depolarizing response (not shown).

The phosphodiesterase inhibitor theophylline applied extracellularly in the concentration 0.5 mM, or injected intracellularly, increased the amplitude and duration of both cAMP and cGMP responses approximately 1.5-fold in 15 neurones out of 23 (fig. 1). At a higher concentration (1 mM) theophylline itself elicited apparent membrane depolarization.

It can be assumed that the main ionic mechanism of cAMP and cGMP depolarization is an increase in potential-independent Na^+-conductance. The following data support this suggestion; R_{in} is decreased during depolarization, the reversal potential of the effect is near −10 mV and finally, the effect is eliminated by the removal of Na^+ from the extracellular solution. The value of the reversal potential also allows us to suggest an involvment of the changes in K^+-conductance, but of course as an additional (not primary) component. At the same time the potential-independence of the effect excludes the participation of Ca^{2+}-channels.

The results of the present study, together with data from the literature[1-9] show that in different cells cyclic nucleotides may regulate the activity of different ionic channels, in different ways. Moreover, the effect of cyclic nucleotide injection into the same identified neurone of different preparations is not always the same. Obviously, cell responses depend on the experimental conditions and the state of the animal.

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0014-4754/85/020252-03$1.50 + 0.20/0

Calcium uptake by sarcoplasmic reticulum from nerve-intact and standard skeletal muscle grafts

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Summary. A freely grafted rat soleus muscle exhibits a decrease in velocity and capacity of SR calcium uptake. This deficit is not prevented by maintaining neural connections (nerve-intact graft) during grafting. Thus the greater mechanical capability of nerve-intact grafts, relative to standard grafts, is not accompanied by any enhancement of the SR tubules.
Key words. Rat soleus muscle; muscle, rat soleus; sarcoplasmic reticulum; muscle graft; calcium uptake.

The sarcoplasmic reticulum (SR) of fast-twitch extensor digitorum longus (EDL) muscles has a faster rate of calcium uptake and a greater capacity for accumulating calcium than does the SR from slow-twitch soleus (SOL) muscles[1,2]. Cross-innervation can reverse the SR calcium uptake properties[3].

When a muscle is severed from its neurovascular supply, it first degenerates due to ischemia, then myogenic cells arise and bring about the regeneration of new muscle fibers[4,5]. If the nerve is not cut during grafting, the resulting nerve-intact graft will also undergo degeneration and regeneration but ultimately possesses a greater muscle mass and can generate a stronger force of contraction than can a standard graft in which both vessels and nerves are severed[6,7]. In view of the neural influence on SR, it was decided to compare calcium uptake by SR in nerve-intact and standard grafts to determine if differences in SR characteristics could be contributing to the observed contractile differences.

Materials and methods. Male Sprague-Dawley rats, weighing an average of 404 ± 10 g when sacrificed, were used in these experiments. Under chloral hydrate anesthesia (40 mg/100 g b.wt) the SOL muscle was exposed and the proximal and distal tendons cut. In one group of rats the vessels to the SOL muscle

Muscle weight and SR calcium uptake in normal and grafted soleus muscles

	Muscle weight (mg)	Calcium uptake Velocity (μmol/mg/min)	Capacity (μmol/mg)	Velocity-capacity ratio (min^{-1})
Control SOL (6)	172.0 ± 7.0[a]	0.089 ± 0.008[a]	0.261 ± 0.014[a]	0.344 ± 0.037[a]
Standard SOL Graft (8)	83.9 ± 14.9[b]	0.052 ± 0.001[b]	0.158 ± 0.010[b]	0.326 ± 0.036[a]
Nerve-intact SOL Graft (9)	128.4 ± 16.2[a,b]	0.052 ± 0.004[b]	0.158 ± 0.007[b]	0.327 ± 0.016[a]

Values are means ± SE. The number of muscles per group is given in parenthesis. Within each column, means with different superscripts are significantly different.