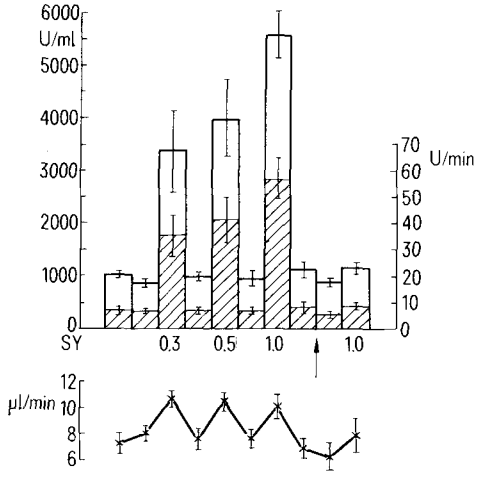
861±71 to 3374±748 units/ml ($p < 0.02$) (mean±SEM), and at 1.0 Hz it rose from 943±116 to 5573±425 units/ml ($p < 0.001$), i.e. to a value similar to that obtained when the sympathetic nerve was excited at 10 Hz in the absence of parasympathetic background activity[9].

Sympathetic stimulation also increased the salivary flow rate. At 0.3 Hz it rose from 8.1±0.5 to 10.7±0.5 µl/min ($p < 0.01$). The higher frequencies did not further accelerate the flow, probably because of increased sympathetic vasoconstriction. Owing to the increased flow rate during sympathetic stimulation, the output of amylase calculated per unit of time rose even more than the concentration, at 0.3 Hz from 7.0±0.8 to 35.6±7.7 units/min ($p < 0.02$).

The β_1-adrenoceptor blocking drug atenolol decreased the amylase secretion in response to sympathetic stimulation. Previous observations indicate that the β-adrenoceptors of salivary glands belong to the β_1-type[10-14], and in rabbits amylase secretion on sympathetic stimulation is abolished by atenolol[4]. In the present experiments, a small response to stimulation at 1.0 Hz remained after atenolol; the dose of the drug was very likely insufficient for complete block.

In experiments carried out in this laboratory on normal rats of the same strain as the one used here, a sympathetic stimulation frequency of 0.5–1.0 Hz was required to produce a perceptible flow of saliva[15]. The present observations show that by providing a low parasympathetic background activity it is possible to obtain at lower frequencies not only secretion of fluid but also a very marked output of amylase; and already at 1.0 Hz, the saliva thus produced contains as much amylase as that secreted at 10 Hz when the gland is activated exclusively via the sympathetic pathway. As shown originally by Ohlin[16], alimentary reflexes in salivary secretion in rats engage not only parasympathetic, but also sympathetic secretory nerves. Hence it can be concluded that the augmented responses[2,3] demonstrated here, when the 2 types of nerves co-operate, are of physiological significance.



Effects of sympathetic stimulation (SY) on the amylase concentration (open columns) and output/min (hatched columns) during parasympathetic degeneration secretion. Below: salivary flow rate. Arrow: atenolol, 2 mg/kg. Means±SEM. When stimulation samples at 0.3, 0.5, 1.0 Hz and 1.0 Hz after atenolol are compared with their preceding controls, the following p-values are obtained: less than 0.02, 0.01, 0.001 and 0.05 for amylase/ml; less than 0.02, 0.02, 0.001 and 0.01 for amylase/min; and less than 0.01, 0.05, 0.05 and 0.05 for flow rates.

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2 N. Emmelin and P. Gjörstrup, Archs. oral Biol. *21*, 27 (1976).
3 N. Emmelin and P. Gjörstrup, Q. Jl exp. Physiol. *60*, 325 (1975).
4 P. Gjörstrup, J. Physiol. *282*, 10P (1978).
5 Ursula Delfs and N. Emmelin, Q. Jl exp. Physiol. *64*, 109 (1979).
6 L.H. Schneyer, J.A. Young and Charlotte A. Schneyer, Physiol. Rev. *52*, 720 (1972).
7 J.R. Garrett, A. Thulin and Audry Kidd, Cell Tiss. Res. *188*, 235 (1978).
8 A. Dahlqvist, Scand. J. clin. Lab. Invest. *14*, 145 (1962).
9 J.R. Garrett and A. Thulin, Cell Tiss. Res. *159*, 179 (1975).
10 J. Ekström, Experientia *25*, 372 (1969).
11 A. Thulin, Experientia *28*, 420 (1972).
12 F.R. Butcher, J.A. Goldman and M. Nemerowsky, Biochim. biophys. Acta *392*, 82 (1975).
13 P. Gjörstrup, Experientia *31*, 550 (1975).
14 B. Carlsöö, Å. Danielsson and R. Henriksson, Br. J. Pharmac. *62*, 364 (1978).
15 A. Thulin, Acta physiol. scand. *96*, 509 (1976).
16 P. Ohlin, Q. Jl exp. Physiol. *53*, 19 (1968).

Naloxone selectively blocks dopamine response of Br-type neuron in *Helix pomatia* L.[1]

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Summary. The present study demonstrates that the potent opiate antagonist, naloxone can selectively block the DA induced inhibition of the bursting activity pattern of the RPal or Br-type neuron. The dopamine inhibitory affect can also be blocked by haloperidol, a established dopamine receptor blocker.

Previous investigations dealing with the molluscan CNS have demonstrated the the physiological and pharmacological characteristics of this system are comparable to the mammalian CNS[3-11]. In *Helix pomatia* the giant identified bimodal pacemaker, Br-type neuron has been shown to receive synaptic inputs[12]. It has also been demonstrated that this neuron is sensitive to the application of various monoamines[12]. Recently, Fuxe et al.[14] reported that naloxone may block dopaminergic transmission in a mammalian system. Therefore, the present study was undertaken to determine if this latter effect exists on a known dopamine responsive cell.

Preparation. Experiments were carried out on the Br-type cell of the snail *Helix pomatia* L. Previously this neuron was termed RPal[15]. The preparation and recording technique have been well documented[13]. Briefly, while being perfused the ganglionic complex was freed from the connective tissue surrounding it. The preparation was then transferred to a 20 ml glass vessel with a open front end so that the cells in question can be identified, positioned and

impaled with the aid of a stereoscopic microscope. The intracellular activity was recorded with glass microelectrodes filled with 2.5 MKCl. Their resistance varied between 8 and 25 MΩ. Recordings were made by the use of a high input impedance amplifier coupled to a four channel tektronix oscilloscope and a brush recorder.

Results and observations. Dopamine application to the cell surface of the Br-type neuron was made perfusion. Control preparations exhibit bursting activity in a very regular and predictable manner. The application of 0.05 µg/ml of DA (figure 1A) resulted in alteration of the normal bursting activity pattern. Within a min the burst frequency decreased. In addition there was a progressive increase in the interburst intervals which was followed by a short period of spike activity elimination. This period of no activity lasted about 1-2 min, then action potentials reappeared but the typical bursting activity was masked by spikes evoked by EPSP (figure 1, B and C). Also, during interburst period synaptic activity could be observed. No changes in spike amplitude or duration were observed. The application of a higher dose of DA (0.5 µg/ml) produced immediate inhibition of bursting activity for a prolonged period of time (4 min) (figure 1, D and E). Toward the end of this long lasting inhibition period, EPSP's were observed followed by some generation of action potentials. All effects caused by DA could be washed out with relative ease. These findings are in agreement with earlier experiments performed on this cell[13].

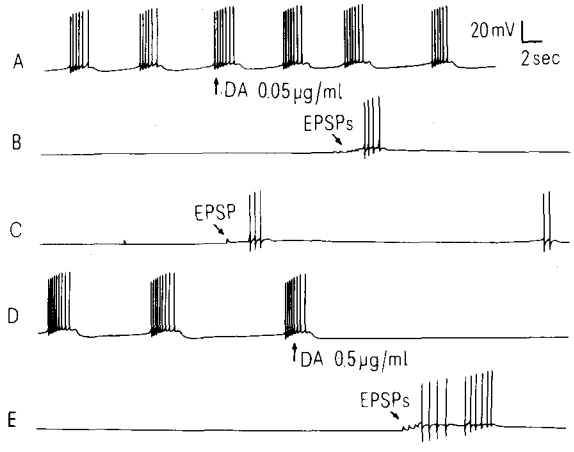

Fig. 1. Effect of dopamine on the Br-type neuron of the isolated *Helix ganglia*. *A* Control activity and application of DA (↑ DA) in a final concentration of 0.05 µg/ml. *B* and *C* 1 min pattern, long lasting inhibition between the abortive bursts and appearing the EPSPs before the burst. *D* Control activity, application of DA (↑ DA) in a final concentration of 0.5 µg/ml. *E* Long lasting inhibition of the spontaneous burst activity accompanied by EPSPs.

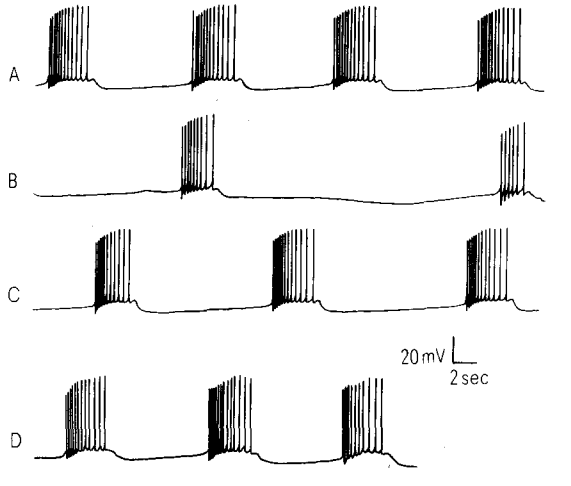

Fig. 2. Effect of dopamine and naloxone on the Br-type neuron. *A* Control activity. *B* Effect of 0.5 µg/ml DA and 0.05 µg/ml naloxone 2 min after application. *C* Effect of 0.5 µg/ml DA and 0.5 µg/ml naloxone 5 min after application. *D* Effect of 0.5 µg/ml DA and 1 µg/ml holoperidol 5 min after application.

The potent opiate receptor blocker naloxone (0.05 µg/ml and 0.5 µg/ml) when applied to the preparation caused no visible effects. Control activity was indistinguishable from naloxone treated preparations.

Co-administration of DA (0.5 µg/ml) and naloxone (0.05 µg/ml) (figure 2, B) resulted in increasing the duration of the hyperpolarization phase of slow waving and a decrease in the number of spikes within burst. The interbursting periods were also increased. Increasing the dose of naloxone 10× completely inhibited the dopamine effect (figure 2, C).

It was also of interest to test the cells response to the blocking action of a known DA antagonist such as haloperidol[16]. Haloperidol administered alone, in concentrations 10-0.01 µg/ml, did not alter the bursting activity during the observation period. Prior treatment of the cell with haloperidol was able to completely block the DA inhibitory response (figure 2, D). This antagonism was first observed at 0.01 µg/ml of haloperidol followed by 0.5 µg/ml DA. It was complete at the 1 µg/ml dose of haloperidol followed by 0.5 µg/ml DA. The data suggests that the DA receptor mechanism present on this cell is more sensitive to naloxone than haloperidol.

Naloxone did not interfere with the norepinephrine or serotonin effect previously described[13]. Therefore naloxone appears to block dopamine in a very selective manner, and at a site also sensitive to haloperidol.

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3 L. Hiripi, Annal. Biol. Tihany *39*, 13 (1972).
4 J. Salanki, L. Hiripi and J. Nemcsok, J. Interdisc. Cycle Res. 5, 277 (1976).
5 L. Hiripi, V. Neuhoff and N.N. Osborne, J. Neurochem. *26*, 335 (1976).
6 G.B. Stefano and E. Aiello, Biol. Bull. *148*, 141 (1975).
7 G.B. Stefano, E.J. Catapane and E. Aiello, Science *194*, 539 (1976).
8 G.B. Stefano, E.J. Catapane and J.M. Stefano, Biol. Bull. *153*, 618 (1977).
9 G.B. Stefano and E.J. Catapane, J. Pharmac. exp. Ther. *203*, 449 (1977).
10 G.B. Stefano, L. Hiripi and E.J. Catapane, J. Therm. Biol. *3*, 79 (1978).
11 E.J. Catapane, G.B. Stefano and E. Aiello, J. exp. Biol. *74*, 101 (1978).
12 J. Salanki, I. Vadasz and K. Elekes, Acta physiol. hung. *42*, 249 (1972).
13 J. Salanki and I. Vadasz, Acta physiol. hung. *44*, 51 (1975).
14 K. Fuxe, L.F. Agnati, P. Bolme, T. Hökfelt, P. Lidbrink, A. Wungdahl, M. Perez-Mora and S. Obnen, in: Mechanisms of Action of Benzodiazepines, p.45. Ed. E. Costa and P. Greengard. Raven Press, New York 1975.
15 D.A. Sakharov and J. Salanki, Acta physiol. hung. *35*, 19 (1969).
16 B.S. Bunney and G.K. Aghajanian; in: Pre and Postsynaptic Receptors, p.89. Ed. E. Usdin and W.E. Bunney. Marcel Dekker Inc., New York 1975.