Effects of dopamine antagonists on snail locomotion

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Summary. Dopamine antagonists which affect the ergometrine-sensitive type of dopamine receptors produce characteristic motor disorders in a land snail, *Helix pomatia* L.

Land pulmonates (snails and slugs) move forward due to coordinated waves of muscular contractions that pass along the sole of the foot. A wave begins at the posterior margin of the sole and moves anteriorly, several consecutive waves being present at a time. This stereotypic motor pattern is a basic part of the crawling locomotion controlled by sensory input. We report here that drugs affecting a specific type of neural dopamine (DA) receptors cause highly reproducible motor disorders in land snails: they activate and disinhibit the contraction wave generator and produce dramatic changes in the animal's posture.

Although the classification of DA receptors is still controversial, even in mammals, the existence of a specific type of DA receptor antagonized by ergometrine, ergotamine and related lysergic acid derivatives, along with 'classic' DA receptors antagonized by butyrophenones and phenothiazines, is nowadays widely recognized. It is believed that in the mammalian brain these 'non-classic' DA receptors are associated with synaptic inhibition[1], as they are in the snail brain, where they were first discovered[2,3].

We used ergometrine and ergotamine as standard antagonists of inhibitory DA receptors. The study was carried out on snails *(Helix pomatia)* collected locally in Tihany, Hungary.

Solutions of alkaloids in 10^{-5}–10^{-3} moles/l concentrations were prepared with snail Ringer[4]. Volumes ranging from 0.05 to 0.8 ml were injected into the body cavity of snails weighing about 20–25 g.

Within 1 min after injecting of ergometrine or ergotamine both frequency and speed of the waves increased. In snails withdrawn into the shell the injection triggered locomotion. At the same time inhibitory influences on the waves disappeared. In normal snails, sudden tactile stimuli, such as touching the head, as well as detaching the sole from the substrate, effectively inhibit wave generation. In treated snails, however, locomotion did not stop at sensory stimulation and, moreover, the waves were propagated along unattached sole (compare figures 1,a, and 2,a). Thus, early manifestation of the ergot effect was pronounced locomotive hyperactivity associated with disinhibition of the mechanism by which the waves are generated. The permanent activity of the wave generator persisted for hours.

Further motor disorders however made it difficult for a snail treated with an alkaloid to move forward. First, the lateral edges of the foot were lifted off the substrate, then the portion of the sole detached from the substrate progressively increased (fig. 2, b) so that finally effective crawling locomotion ceased, although the waves were generated and

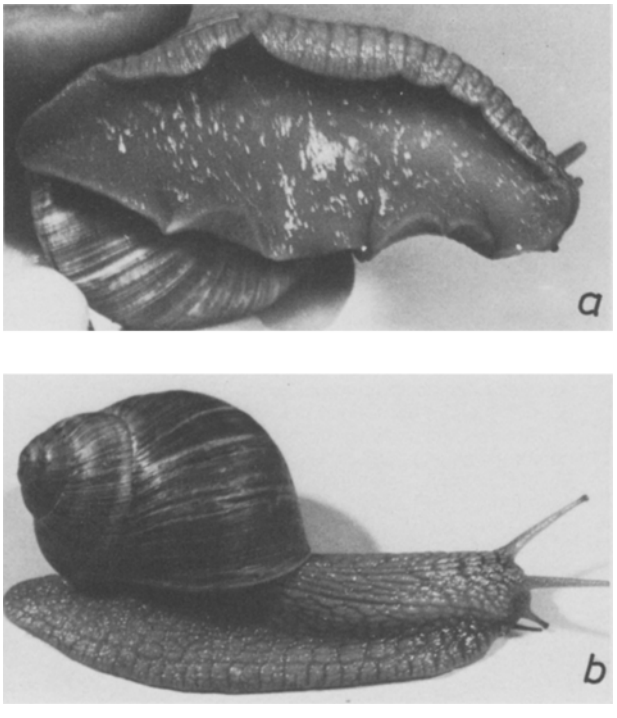

Figure 1. Normal snail *Helix pomatia* L. *a* Appearance of the sole detached from the substrate during spontaneous crawling locomotion. Surface of the sole is smooth, peripheral edges are bending inward. *b* The crawling snail.

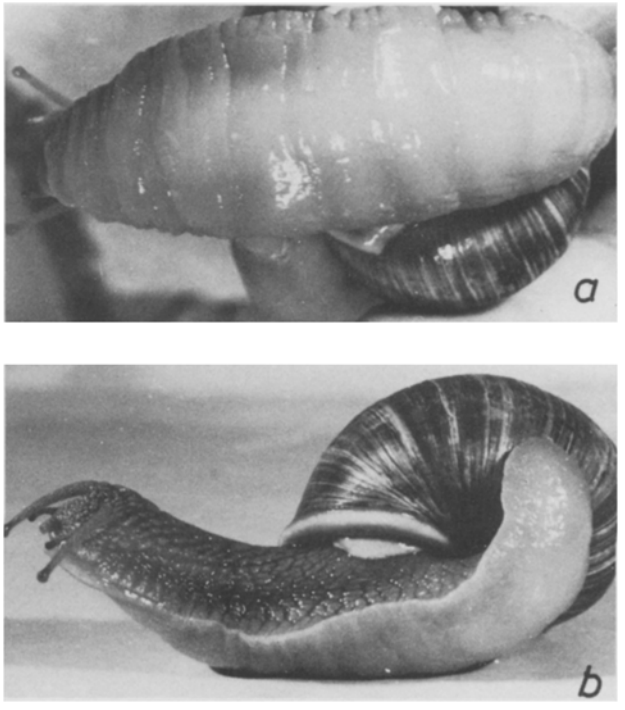

Figure 2. The same snail 2 (a) and 7 (b) min after administration of ergometrine (0.1 ml of the mmole/l solution). *a* Appearance of the sole detached from the substrate during locomotion activated by the injection. Notice waves of muscular contractions on the surface of the sole and changed position of its peripheral edges. *b* Disabled snail on the substrate. A significant part of the sole is characteristically out of contact with the substrate, waves of contractions are seen in its lower part.

movements of the foot occurred. We suggest for this behavioral effect the term 'sole detachment'.

The minimum dose of ergometrine or ergotamine producing this effect was 0.4 ml of a 10^{-4} moles/l solution per snail. Lower doses resulted only in hyperactivity; sole detachment did not develop. The 'sole detachment' effect appeared within some min to 1 h depending on the magnitude of the dose. Most snails injected with up to 0.5 ml of a 10^{-3} moles/l solution recovered completely, but larger doses were lethal.

To determine, whether these effects were drug-specific, we investigated behavioral effects of a number of drugs which block DA receptors or otherwise affect synaptic transmission. 4 constant volumes of each of the drugs (0.1, 0.2, 0.4, and 0.8 ml) were injected into the body cavity of snails.

The following substances were used as 10^{-3} moles/l standard solutions dissolved in snail Ringer: haloperidol, chlorpromazine (standard mammalian DA antagonists of the butyrophenone and phenothiazine series, respectively), d-tubocurarine, strychnine (these 2 drugs reportedly eliminate the excitatory responses of snail neurons to DA)[5], methysergide (the most effective antagonist of ergometrine-sensitive serotonin receptors in snails)[6], bromo-LSD (D-2-bromolysergic acid diethylamide), phentolamine, hexamethonium, atropine, tetraethylammonium, morphine, dopamine, serotonin, octopamine, glutamate, acetyl-choline.

Of all the drugs used, only bromo-LSD produced effects which were virtually identical to those of ergometrine and ergotamine. In a previous study we observed similar motor disorders in snails treated with 6-hydroxydopamine[7]. Some of the drugs used in the present study (among them phentolamine, haloperiodole and, notably, serotonin) produced hyperactivity; they could not, however, elicit 'sole detachment'. The results show that hyperactivity alone is not a reliable manifestation of blockade of ergometrine-sensitive DA receptors. Combined with 'sole detachment' and disinhibition, hyperactivity however seems to manifest specific and reproducible behavioral effects of the DA antagonists.

DA is an established neurotransmitter in molluscs capable of exerting both excitatory and inhibitory synaptic effects[6]. Derivatives of lysergic acid such as d-LSD, ergometrine and ergotamine were shown to be the most potent agents affecting DA-sensitive binding of a ^{3}H-labelled ligand to the particulate fraction of *Helix* ganglia[8]. The same substances, as well as 6-hydroxydopamine[9], are the most potent antagonists of hyperpolarizing effects of DA on gastropod neurons[2,3,5,6].

It is obvious from the present results that these agents have a similar exclusive potency in the behavioral effects described here. We conclude that the receptors in question play an important part in the mechanisms controlling locomotion and posture in land pulmonates. This conclusion is in accordance with data from other molluscs which indicate a role of DA in motor control[10-13]. Our results do not necessarily imply that ergometrine-sensitive serotonin receptors are not involved in the above behavioral reactions, although the fact that 6-hydroxydopamine produced the motor disorders while methysergide did not, makes this involvement unlikely. Characteristic and reproducible motor disorders produced by DA antagonists in snails make it possible to use these animals as a simple animal model for screening compounds that affect the specific type of DA receptors.

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The pharmacokinetics of VIP in dog and pig

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Summary. Pure porcine VIP was infused systemically in 4 conscious dogs and systemically and intraportally in 6 anesthetized pigs. At 2.3 pmoles · kg^{-1} · min^{-1} the MCR was 10.7±1.0 ml · kg^{-1} · min^{-1} in dog and 7.6±1.5 (systemic) and 16.5±2.0 (portal) in pig. The $t_{1/2}$'s were 1.0±0.2, 0.85±0.12 and 1.0±0.05 respectively. These values agree with those observed in man. This very high single pass tissue clearance does not suggest a hormonal role for VIP.

VIP has been isolated from the hog small intestine[2] and more recently from the CNS in large quantities[3-5]. It has a wide range of pharmacological actions including powerful vasodilation[2] and potent stimulation of small intestinal secretion[6]. The localization of VIP in fine nerve fibers in the peripheral tissues is in accord with its probable action as a neurotransmitter or neuromodulator[7]. Few physiological stimuli have been shown to cause a significant rise in peripheral plasma VIP but changes are seen after mesenteric ischaemia[8] and vagal stimulation in the calf[9] and pig[10]. A role as a circulating hormone thus cannot be completely excluded. In this study the systemic pharmacokinetics of the dog and pig were investigated and compared with data on the pharmacokinetics of VIP in man[11].

Materials and methods. Four trained conscious mongrel dogs (weight range 12–16 kg) in tight harness restraints were given infusions of VIP or saline in random order on separate days. After an overnight fast the femoral vein was