RASSEGNE

On the mode of action of *Latrodectus* spp. (*) venom (**)

SERGIO BETTINI

Department of Parasitology

Summary. — The literature on the pharmacological and electrophysiological effects of *Latrodectus* spp. venom, obtained by different methods, on vertebrates and invertebrates (arthropods) is listed. Recent unpublished findings on the venom effects on vertebrate neuromuscular junctions and on arthropod afferent nerve junctions are reported. It is pointed out that, in arthropods, all sites where the venom has been proven to be active are ACh-sensitive. Nor can it be excluded that latrodectism symptoms and pharmacological effects on vertebrates may be due to the action on cholinergic sites only.

Riassunto (*Sul meccanismo d'azione del veleno di* Latrodectus *spp.*). — Vengono riferiti i dati, riportati in letteratura, sugli effetti farmacologici ed elettrofisiologici del veleno di *Latrodectus* spp., ottenuto con varie tecniche, sui vertebrati ed invertebrati (artropodi).

L'Autore quindi descrive i risultati, non ancora pubblicati, osservati in seguito all'effetto del veleno sulle giunzioni neuromuscolari di vertebrato e sulle giunzioni nervose di artropodo. Viene sottolineato il fatto che negli artropodi tutti i siti, ove il veleno si è manifestato attivo, sono sensibili all'ACh. Non può essere escluso, secondo l'Autore, che i sintomi da latrodectismo e gli effetti farmacologici sui vertebrati possano essere dovuti all'azione sui soli siti colinergici.

(*) Most of the research work reported in the present paper was carried out on *Latrodectus mactans tredecimguttatus* and *Latrodectus mactans mactans*.

(**) Presented at the 2nd International Symposium on Animal and Plant Toxins, Tel Aviv, February 22-28. 1970. From this date the following papers have been published: BETTINI, FRONTALI & GRASSO, CLARK *et al.*, D'AJELLO, MAGNI & BETTINI, LONGENECKER *et al.*, MAROLI & BETTINI; the paper of MAJORI & BETTINI has been accepted for publication. The text and bibliography has been modified accordingly.

The present paper deals with a brief review of the findings, many of which still unpublished, on the mode of action of black widow spider venom and includes a discussion, in the light of these observations, of latrodectism symptomatology.

The experimental work on *in vitro* vertebrate preparations dates back about 50 years, while that on invertebrates (arthropods) is about 5 years old. All experiments reported here, unless otherwise stated, were carried out by using crude venom gland extracts. The greatest part of the research has been devoted to the nervous and muscular systems of both vertebrates (BETTINI & CANTORE, 1959) and invertebrates. However, a few other vital systems have been explored and the following results obtained. The venom shows a marked hyaluronidase effect (CANTORE & BETTINI, 1958*a*), but no anti-acetylcholinesterase (AChE) activity (BETTINI & TOSCHI-FRONTALI, 1960); its cytotoxic activity on vertebrate cells cultivated *in vitro* is strikingly high (VICARI *et al.*, 1965).

Arthropod preparations

Latrodectus venom is highly toxic to arthropods (BETTINI & TOSCHI-FRONTALI, 1960; GRASSO & PAGGI, 1967). This observation has encouraged research on nerve and neuromuscular preparations of crustacea and insects.

The effects of the venom on the spontaneous electrical activity recorded across the mesothoracic ganglion of the cockroach *Periplaneta americana* are characterized by an increase in spike frequency followed immediately after by block of the activity (NERI, BETTINI & FRANK, 1965).

Two nerve junctions of the cockroach have also been investigated: the cercal nerve-giant neurons and the giant neurons-N5 of the metathoracic ganglion. They are both affected by the venom which induces a transient increase of spontaneous activity, followed by its block, and failure of induced synaptic transmission. It has also been demonstrated that the venom does not alter axonic conduction (D'AJELLO, MAURO & BETTINI, 1969; MAROLI & BETTINI, 1971).

Recently, the effects of the venom have been more clearly shown by intracellular electrode investigations. Here too the venom determines failure of response in the giant neurons, even at higher voltage impulses, following orthodromic stimulation by the cercal nerves, and a gradual decrease of excitatory postsynaptic potential. Along with this phenomenon, the venom induces a transient burst of spontaneous activity, but does not block the response to antidromic stimulation (D'AJELLO, MAGNI & BETTINI, 1971). In discussing the mode of action of the venom, the authors point out that an eventual acetylcholinomimetic action is highly improbable owing to the high molecular weight of the toxic proteins [higher than 50,000,

according to FRONTALI & GRASSO (1964), and of about 5,000, according to MC CRONE & HATALA (1967)]. That the venom possesses no anti-AChE activity has already been demonstrated by BETTINI & TOSCHI-FRONTALI (1960). The hypothesis put forward by D'AJELLO, MAGNI & BETTINI (1971) is that the venom acts on the presynaptic membrane inducing a sustained release of ACh and, consequently, a depolarization of the postsynaptic membrane.

The venom also induces on the stretch receptor of the crayfish *Astacus astacus* an increase of impulse frequency followed by block of activity GRASSO & PAGGI, 1967).

The cockroach semi-isolated heart preparation reacts at very low venom concentrations; it causes first an increase of frequency of the heartbeat and then its block (MAJORI & BETTINI, in press).

Finally, *Latrodectus* venom has been tested on two neuromuscular preparations: the venom obtained by electrical extraction, on the motor nerve-deep abdominal *extensor medialis* muscle of the crayfish *Procambarus clarki* (PARNAS & RUSSELL, 1967) and the gland extract on nerve 4-extensor of the trochanter (metathorax) of the cockroach (MAROLI & BETTINI, 1971). No effect on the neuromuscular junction or on the muscle of either preparation was noted.

It appears, therefore, that the venom acts on the nerve junction of these arthropods where ACh has been indicated as being the probable chemical transmitter or where at least the nerve junction has been demonstrated to be sensitive to ACh (BOISTEL, 1968), namely, at the 6th abdominal ganglion (transmission between cercal fibres and giant fibres) and at the 3rd thoracic ganglion (transmission between giant neurons and leg motor fibres), and at the cardiac ganglionic cells whose spontaneous activity is extremely sensitive to cholinergic compounds, although no evidence has been found for a cholinergic synapse in the cardiac nervous system (MILLER, 1968).

On the other hand, the venom seems to have no effect on the arthropod neuromuscular junctions, which have repetitively been demonstrated to be ACh-insensitive (KERKUT & WALKER, 1966).

Vertebrate preparations

Several authors, by employing different techniques, have shown that the venom causes a marked bronchospasm in the guinea pig (HOUSSAY & NEGRETE, 1919; KELLEWAY, 1930; SAMPAYO, 1944; CANTORE & BETTINI, 1958*b*).

It was also demonstrated that the tonus of the isolated rabbit ileum increases significantly while its rhythmic spontaneous contractions are unaffected by the venom (SAMPAYO, 1942; CANTORE, 1958).

Experiments have been conducted by several authors on neuromuscular preparations. As far back as 1929, TROISE observed that an evident fibrillation was induced on frog *sartorius* muscle bathed in venom solution.

By employing a rat neuromuscular preparation (not better identified by the author), D'AMOUR, BECKER & VAN RIPER (1936) reported that the venom showed no effect on the nerve trunk, the muscle or the neuromuscular junction. Negative results were also obtained by SAMPAYO (1944) on toad *sartorius* neuromuscular preparation and on the denervated muscle.

Research carried out by TROISE (1928) and more fully by SAMPAYO (1942) on anaesthetized and on spinal animals, led the authors to conclude that the venom acted on the cerebral cortex (EEG modifications) and on the spinal cord (exaggeration of muscular sympotms in spinal animals), but not on motor nerves or on muscles.

On the other hand, other authors using the phrenic nerve-diaphragm preparation of rat (CANTORE, 1958) or of guinea pig (RUSSELL & LONG, 1961; RUSSELL, O'BRIEN & INABA, 1961), where the nerve was electrically stimulated, found that the venom caused a definite neuromuscular block.

These findings have been recently substantiated. Intracellular recordings of frog *sartorius* muscle fibres have shown that the venom induced very high rates of minipotential release at zero calcium concentration. Through electron microscope pictures, it has also been demonstrated that the nerve endings, following the minipotential release, were completely depleted of ACh vescicles (CLARK *et al.*, 1970; LONGENECKER *et al.*, 1970).

Recordings through transducer of the dynamic effects of venom on frog muscles (MAROLI & BETTINI, 1971) have shown that a series of fibrillations and fasciculations precedes the neuromuscular block and that this phenomenon is accompanied by increased muscle tension, lasting about 30 minutes. Eserine strongly potentiates fibrillation and fasciculation. Furthermore, frog leg muscles bathed in venom solution, were subsequently washed and kept in saline + 10^{-5} g/ml eserine for 40 minutes. This washing fluid, in contact with the abdominal *rectus* muscle, produced a contracture comparable to that obtained on the same muscle by 10^{-8} g/ml ACh.

Let us now list the main symptoms due to *Latrodectus* bite in man and see whether any correlation may be drawn with the above reported observations.

The symptoms may be separated for convenience into three main groups (BETTINI & CANTORE, 1959). The first one involves the CNS: psychomotor excitation, hallucinations, confusion, etc. make up the well known and very dramatic psychic picture which, with no exception, accompanies latrodectism cases.

The second group is related to the voluntary muscles and their motor nerves. It includes spastic muscular contractions, tremors and contractures.

The third group, which is by far the largest, is related to symptoms connected with the autonomic NS. Mydriasis and contraction of cat's nictidating membrane, constipation, hypertension, (CANTORE & BETTINI, 1958*c*) perspiration, are all symptoms to be referred to stimulation of the sympathetic pathways. All other autonomic symptoms are related to the parasympathetic pathways. They include lacrimation, vomiting, bronchospasm, urine retention and prispism. On the other hand, symptoms of both cholinergic and adrenergic origin may appear: excessive salivation (1,500 ml in 24 hours) or dry mouth; bradycardia or tachycardia.

In the light of the above reported pharmacological and neurophysiological results, two closely related mechanisms may be advanced to explain the mode of action of *Latrodectus* venom.

The first hypothesis suggests that the venom possesses a selective action at cholinergic presynaptic membranes, and would account for all cholinergic effects which appear at different levels· CNS symptoms, post ganglionic effects on autonomic cholinergic terminals (muscarinic effect) and ganglionic effect (nicotinic effect) with consequent cholinergic and adrenergic effects.

It would fit into this picture that atropine, though acting as a venom antagonist on the isolated ileum, shows no effects at the level of ganglia; its possible anti-muscarinic effect would manifest itself through a potentiation of the venom adrenergic effects (hypertension, perspiration, mydriasis, etc.). This occurs in experimental latrodectism treated with atropine. Therefore, the absence of antidotic effects of atropine in human poisoning (SAMPAYO, 1942) would be justified.

The fact that the extirpation of the superior cervical ganglion suppressed mydriasis and contraction of the nictidating membrane (SAMPAYO, 1944), would also fit this hypothesis.

As second hypothesis, it is assumed that ACh massively released at the neuromuscular junctions and not coped with by AChE, could be directly taken into the blood stream. Should this be the case, the venom, besides the direct muscular effects, would also induce all the cholinergic symptoms reported for the first hypothesis.

Furthermore, owing to the increased permeability of the blood-brain barrier, as shown by MARETIC & JELASIC (1953) in cats bitten by *Latrodectus*, ACh could reach the CNS and induce there the EEG modifications and the characteristic psychic symptoms. According to this mechanism, ACh could eventually affect also the afferent endings, e.g. sweat response (COON & ROTHMAN, 1939); nicotinic action in nerves of the mesentery (BROWN & GRAY, 1948).

Finally, it should be pointed out that death by subcutaneous injection of ACh is determined by bronchospasm and pulmonary oedema, which are constantly found also in experimental animals killed by the venom.

The first hypothesis could very well account for a common mode of action of the venom on both arthropods and vertebrates, while the second hypothesis could not possibly explain the toxic picture presented by arthropods since their neuromuscular junctions are not cholinergic.

Further evidence is needed to solve the mode of action of this venom. The problem becomes more complex when we consider that several toxic protein fractions have been separated, showing selective activities according to animal group and to organ (BETTINI, FRONTALI & GRASSO, 1970). It would be interesting to investigate to which fraction, or fractions, the activity on the cholinergic vertebrate junctions is due. Since these proteins were obtained from gland extracts, a parallel research with milked venom would also be called for.

Received June 6, 1971.
Accepted July 20, 1971.

REFERENCES

BETTINI, S. & G. P. CANTORE, 1959. Quadro clinico del Latrodectismo. *Riv. Parassitol.*, **20**, 49.

BETTINI, S. & N. TOSCHI-FRONTALI, 1960. Biochemical and toxicological aspects of *Latrodectus tredecimguttatus* venom. *XI Intern. Kongr. Entomol.*, Wien. Verh. B. III. (Symp. 3), p. 115.

BETTINI, S., N. FRONTALI & A. GRASSO, 1970. Recent findings on the biochemistry and toxicology of *Latrodectus mactans tredecimguttatus* venomous glands extract. Presented at the *4th Congr. Eur. Int. Arachnology*, Paris, 1968; published in *Bull. Mus. Natl. Hist. Nat.* 2° Ser., Suppl. 1, **41**, 251, 1970.

BOISTEL, J., 1968. The synaptic transmission and related phenomena in insects. *Advan. Insect Physiol.*, **5**, 1.

BROWN, G. L. & J. A. B. GRAY, 1948. Some aspects of nicotine- like substances and their relation to sensory nerve endings. *J. Physiol.*, **107**, 306.

CANTORE, G. P., 1958. Contributo allo studio dell'azione farmacologica del veleno di *Latrodectus tredecimguttatus* Rossi. *Riv. Parassitol.*, **19**, 158.

CANTORE, G. P. & S. BETTINI, 1958*a*. Contributo allo studio del veleno di *Latrodectus tredecimguttatus* Rossi. *Rend. Ist. Super. Sanità*, **21**, 797.

CANTORE, G. P. & S. BETTINI, 1958*b*. Contributo allo studio dell'azione farmacologica del veleno di *Latrodectus tredecimguttatus* Rossi. II: azione sulla muscolatura bronchiale. *Riv. Parassitol.*, **19**, 197.

CANTORE, G. P. & S. BETTINI, 1958*c*. Contributo allo studio dell'azione farmacologica del veleno di *Latrodectus tredecimguttatus* Rossi. III: azione sul ritmo cardiaco e sul circolo arterioso. *Riv. Parassitol.*, **19**, 301.

COON, J. M. & S. ROTHMAN, 1939. Nature of a sweat response to drugs with nicotine-like action. *Proc. Soc. Exptl. Biol. Med.*, **42**, 231.

D'AJELLO, V., F. MAGNI & S. BETTINI, 1971. The effect of the venom of the black widow spider *Latrodectus mactans tredecimguttatus* on the giant neurones of *Periplaneta americana*. *Toxicon*, **9**, 103.

D'AJELLO, V., A. MAURO & S. BETTINI, 1969. Effect of the venom of the black widow spider, *Latrodectus mactans tredecimguttatus*, on evoked action potentials in the isolated nerve cord of *Periplaneta americana*. *Toxicon*, **7**, 139.

D'AMOUR, F. E., F. E. BECKER & W. VAN RIPER, 1936. The black widow spider. *Quartely Rev. Biol.*, **11**, 123.

FRONTALI, N. & A. GRASSO, 1964. Separation of three toxicologically different protein components from the venom of the spider *Latrodectus tredecimguttatus*. *Arch. Biochem. Biophys*, **106**, 213.

GRASSO, A. & P. PAGGI, 1967. Effect of *Latrodectus mactans tredecimguttatus* venom on the crayfish stretch receptor neurone. *Toxicon*, **5**, 1.

HOUSSAY, B. A. & J. NEGRETE, 1919. Nuevos estudios experimentales sobre la accion fisiologica de las ponsonas de las arañas. *Rev. Inst. Bacteriol. Bs. Aires*, **2**, 189.

KELLEWAY, C. H., 1930. The venom of *Latrodectus hasseltii*. *Med. J. Australia*, **1**, 41.

KERKUT, G. A. & R. I. WALKER, 1966. The effect of l-glutamate, acethylcholine and gamma-amino butirric acid on the miniature end-plate potentials and contractures of the coxal muscles of the cockroach *Periplaneta americana*. *Comp. Biochem. Physiol.*, **17**, 435.

MAJORI, G. & S. BETTINI. Effects of *Latrodectus mactans tredecimguttatus* venom on the heart of *Periplaneta americana* (in press on *J. Insect Physiol.*).

MARETIC, Z. & F. JELASIC, 1953. Über den Einfluss des Toxins der Spinne *Latrodectus treaecimguttatus* auf das Nervensystem. *Acta tropica*, **10**, 209.

MAROLI, M. & S. BETTINI, 1971. Effects of *Latrodectus mactans tredecimguttatus* venom on the dynamics of vertebrate voluntary muscles. *Ann. Ist. Super. Sanità*, **7**, 44.

MC CRONE, J. D. & R. J. HATALA, 1967. Isolation and characterization of a lethal component from the venom of *Latrodectus mactans mactans*. In: *Animal Toxins*, F. E. Russel, & P. R. Saunders Eds., Pergamon Press., Oxford, p. 29.

MILLER, T., 1968. Role of cardiac neurones in the cockroach heartbeat. *J. Insect Physiol.*, **14**, 1265.

NERI, L., S. BETTINI & M. FRANK, 1965. The effect of *Latrodectus mactans tredecimguttatus* venom on the endogenous activity of *Periplaneta americana* nerve cord. *Toxicon*, **3**, 95.

PARNAS, I. & F. E. RUSSELL. Effect of venoms on nerve, muscle and neuromuscolar junction. In: *Animal Toxins*, F. E. Russell & P. R. Saunders Eds., Pergamon Press, Oxford, p. 401.

RUSSELL, F. E., B. A. O' BRIEN & D. I. INABA, 1961. Venoms and neuromuscolar transmission. *Proc. 10th Pac. Sci. Congr.* Honolulu, p. 455.

RUSSELL, F. E. & T. E. LONG, 1961. *Effects of venoms on neuromuscolar transmission. Myastenia gravis* H. R. Viets, Ed., **3**, 101.

SAMPAYO, R. R. L., 1942. « *Latrodectus mactans* » *y latrodectismo*. El Ateneo, Bs. Aires.

SAMPAYO, R. R. L., 1944. Pharmacological action of the venom of *Latrodectus mactans* and other *Latrodectus* spiders. *J. Pharmacol. Exptl. Therap.* **80**, 309.

TROISE, E., 1928. Estudio farmacologico sobre la ponsona del *Latrodectus mactans*. *Compt. Rend. Soc. Biol.*, **102**, 1097.

TROISE, E., 1929. Action de venin de l'araignée *Latrodectus mactans*. *Compt. Rend. Soc. Biol.*, **102**, 1097.

VICARI, G., S. BETTINI, C. COLLOTTI & N. FRONTALI, 1965. Action of *Latrodectus mactans tredecimguttatus* venom and fractions in cells cultivated *in vitro*. *Toxicon*, **3**, 101.