Norepinephrine as principal catecholamine in a specific neurone of an invertebrate (*Boophilus microplus:* Acarina)

B.F. Stone, K.C. Binnington and A.L. Neish

CSIRO, Division of Entomology, Private Mail Bag 3, Indooroopilly (Queensland, Australia 4068), 7 December 1977

Summary. Norepinephrine has been identified as the principal catecholamine in individual neurons of the synganglion of an arthropod, the cattle tick *Boophilus microplus.* This suggests that norepinephrine may have a hitherto unsuspected major physiological role in at least one group of invertebrates.

Dopamine (DA) is considered to be the principal catecholamine (CA) acting as a neurotransmitter in the invertebrate central nervous system (CNS) as there is usually at least 10 times more DA than norepinephrine (NE) present[1]. 1 apparent exception is in the cattle tick *Boophilus microplus* where DA and NE have been extracted from synganglion (CNS) tissue[2] and shown to occur there in almost equal quantities[3]. Both DA and NE have already been identified by the Falck-Hillarp method[4] in neuronal somata and also in varicose fibres of both the synganglion and salivary glands of *B. microplus*[5]. We report here the 1st identification of NE as the principal CA in individual neurones of an identified group in a tick. This result suggests that NE may be a neurotransmitter or neurosecretomotor substance[5] of significance in at least 1 group of invertebrates.

Biochemical methods have been applied to the detection of putative transmitter substances in large invertebrate neurones (approaching 1000 µm in diameter) which are able to be dissected by hand[6]. However, microspectrofluorometry following formaldehyde vapour treatment of tissue[4] was used in this investigation as it is the only method known to us which is sufficiently sensitive to permit identification of CA's in individual neurones as small as those in the CNS of *B. microplus* (about 40×30 µm[5]).

Although the fluorophores produced from both DA and NE have the same excitation and emission maxima, subsequent exposure to HCl vapour produces different excitation spectra[7]. This excitation wavelength peak shift within the visible spectrum forms the basis of a reliable method, verified by tissue model systems, for distinguishing NE from DA in single neurones isolated from the tick synganglion by a smear technique. Protein models were prepared by spraying microscope slides with freshly prepared solutions of bovine serum albumin (10 mg/ml) containing DA or NE or a 1:1 mixture (1 mg/ml-pH 7.0). Tissue models were prepared from synganglia which had been incubated in DA or NE (0.5 mg/ml) in a tick dissection medium (II)[8] for 10 min at room temperature after prior depletion of endogenous catecholamines by 2 injections of reserpine (50 µg/g) into engorged female ticks, the interval between

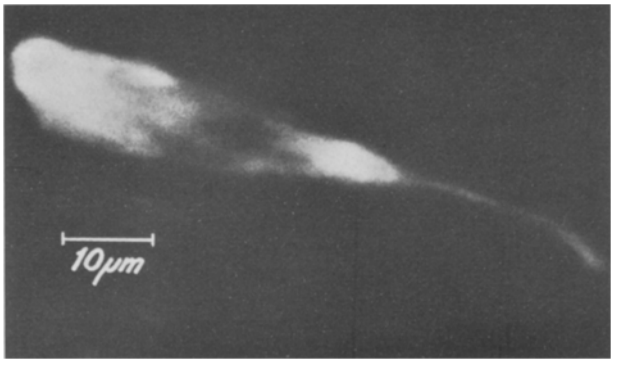

Fig. 1. Large, isolated postero-dorsal neurone of the synganglion of the cattle tick *Boophilus microplus* fluorescing after formaldehyde vapour treatment. Noradrenaline identified in cell bodies of this type.

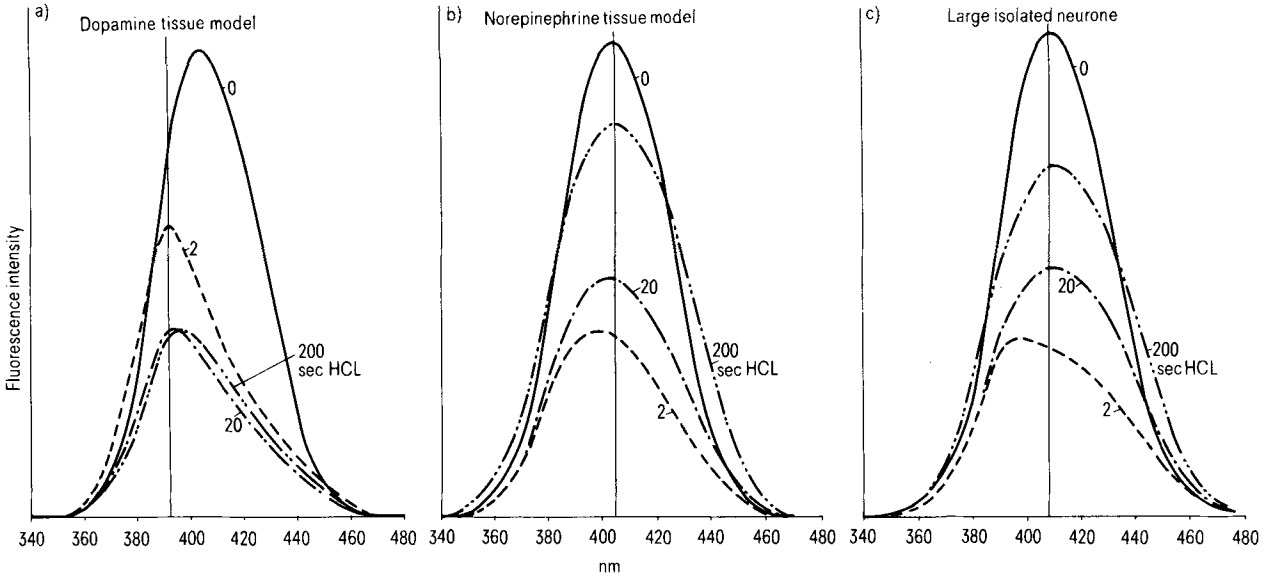

Fig. 2. Excitation spectra of formaldehyde-induced fluorophores of (a) dopamine (DA) and (b) norepinephrine (NE) in tissue models after reserpine depletion of endogenous catecholamines and replacement by DA or NE. Spectra were compared with (c) fluorophores in a large isolated postero-dorsal neurone of the cattle tick *Boophilus microplus.* Sensitivity was unaltered during exposure to HCl (cumulative total exposures of 0, 2, 20, and 200 sec).

injection and dissection being 24 h. All synganglia, after removal of the sheath forming the periganglionic sinus and neurilemma, were smeared very thinly on slides to obtain isolated neurones well separated from surrounding tissue. Preparations were air dried on slides, exposed for 1 h to formaldehyde vapour generated from paraformaldehyde equilibrated to 65±2% rel. humidity and coverslip mounted in paraffin oil. The large postero-dorsal neurones were clearly distinguishable by size from other groups of smaller neurones[5].

Excitation spectra were obtained using a microspectrofluorometer which was similar to other instruments[9] based on the Leitz MPV and equipped with an incident light illuminator and cooled photomultiplier. The spectral half band width was 8.5 nm for the excitation and emission monochromators. Glass optics were adequate since the definitive wavelength shifts occurred above 360 nm. After the initial recording of the spectral curve each preparation was exposed successively to HCl vapour in a Coplin jar for 2, 20 and 200 sec and spectra re-recorded precisely at the same location on each slide. The high voltage to the photomultiplier was kept constant for each preparation[7].

Isolated fluorescent neurones (figure 1) from synganglion smears untreated with catecholamines had emission spectra which were characteristic of either DA or NE fluorophores produced in protein or tissue models; the emission maximum was 480 nm which clearly excluded the visually distinguishable 5-hydroxy tryptamine which has an emission peak of 530 nm. However, after HCl treatment, there was a clear differentiation between DA and NE in protein and tissue models as the DA excitation maximum (408 nm) shifted to a lower wavelength (394 nm) where it remained while the NE maximum after a transitory shift to 395–400 nm moved back to 408 nm. In tissue models in which isolated cells were examined, this move by the NE maximum back to the higher wavelength was accompanied by an increase in intensity after 200 sec exposure. Neurones exposed to HCl vapour in a similar manner to the models had excitation spectra which closely resembled those of NE-treated protein and tissue models; the latter spectra provide the more valid basis of comparison and are therefore included for comparison (figure 2).

These observations demonstrate that NE is present in the larger postero-dorsal neurones of the cortex[5] and is almost certainly the principal catecholamine[10]. Both NE and DA have been extracted from synganglion tissue of *B. microplus*[2,3] and either or both occur in varicose fibres of the neuropile[5]. However, measurement of fluorescence intensity in the small terminals and isolation of these terminals and varicose fibres from surrounding tissue must await even more sensitive techniques.

1 G.A. Kerkut, Br. med. Bull. *29*, 100 (1973).
2 Anon. CSIRO, Div. Ent. Ann. Rep. (1974–75).
3 M.W.J. Megaw and H.A. Robertson, Experientia *30*, 1261 (1974).
4 B. Falck and C. Owman, Acta Univ. lund. II, *7*, 1 (1965).
5 K.C. Binnington and B.F. Stone, Comp. Biochem. Physiol. *58c*, 21 (1977).
6 J. Kehoe and E. Mardor, Ann. Rev. Pharmac. Toxic. *16*, 245 (1976).
7 O. Eränkö and L. Eränkö, J. Histochem. Cytochem. *19*, 131 (1971).
8 W.R. Kaufmann and J.E. Phillips, J. exp. Biol. *58*, 537 (1973).
9 N.G.M. Wreford and G.C. Schofield, J. Microsc. *103*, 127 (1975).
10 Formaldehyde vapour-treated protein models incorporating a 1:1 mixture of DA and NE had excitation spectra closely resembling that of DA so that DA, if present in the isolated neurones, would almost certainly be much lower in concentration than NE.

Seasonal changes in the electrical parameters of the small intestine, colon and bladder mucosa of land tortoises (*Testudo hermanni hermanni* Gmelin)[1]

Michelle Gilles-Baillien and Myriam Verbert

Laboratory of General and Comparative Biochemistry, University of Liège, B-4020 Liège (Belgium), 5 January 1978

Summary. During hibernation of tortoises, a marked decrease in the short-circuit current together with an increase in the electrical resistance are observed across the small intestine, colon and bladder mucosa and D-aldosterone (which is decreased in the plasma) has no effect on these parameters.

It has been pointed out previously that permeability characteristics to inorganic ions of small intestine and colon epithelia are altered during hibernation[2]. Changes in the inorganic ion content of the small intestine, colon and bladder mucosa, as well as in muscle, were also detected[3]. The inexchangeable fraction of intracellular Na and K has also been shown to vary during the winter in the small intestine and colon[4] as well as in the bladder[5]. These changes should be reflected in the electrical parameters of these epithelia.

Each epithelium is cleared of muscular and connective layers by dissection, and set in between plexiglas chambers

Table 1. Potential difference (PD), short-circuit current (SCC) and resistance (R) across small intestine, colon and bladder mucosa of active and torpid tortoises

	PD (mV) Active	Torpid	SCC (μA/cm²) Active	Torpid	R (Ω × cm²) Active	Torpid
Small intestine	1.5± 0.9 (11)	2.6±0.9 (9) S	12.8± 3.3 (9)	4.5± 2.9 (8) HS	117	578
Colon	29.7±13.3 (22)	22.3±11.9 (10) NS	58.6±31.3 (11)	16.0±8.2 (13) HS	507	1394
Bladder	64.8±27.0 (14)	15.3± 4.4 (18) HS	205.3±82.9 (6)	25.2±19.8 (18) HS	316	607

Results are means±SD. Number of individuals between brackets. The probability to the equivalence of the means is given as follows (t-test of Student): S, the change is significant (level 5%); HS, highly significant (level 1%); NS, not significant.