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OCTOPAMINE SENSITIVITY OF THE BLOOD–BRAIN BARRIER OF AN INSECT

BY P. K. SCHOFIELD AND J. E. TREHERNE

AFRC Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, The University, Downing St, Cambridge CB2 3EJ, UK

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SUMMARY

Octopaminergic sensitivity of the glia that form the blood–brain barrier of the cockroach was examined using microelectrode recordings of the potential developed across this barrier layer, the perineurium. Exposure to 10^{-4} mol l^{-1} DL-octopamine had no significant effect upon the changes in potential induced by substitution of Tris for external sodium. Treatment with concentrations ranging from 10^{-4} mol l^{-1} down to 5×10^{-7} mol l^{-1} enhanced the resting level of the potential after 5 min, with an accompanying depression of the magnitude of potassium-induced steps. At 10^{-7} mol l^{-1}, there was no significant effect upon the resting level, but there was a reduction in potassium sensitivity. In a study of the effect of other neurohormonal factors, it was found that DL-synephrine could depress the response to potassium but was less effective than octopamine. In contrast, carbamylcholine, histamine and some compounds related to octopamine enhanced the potassium sensitivity. The effect of octopamine was completely blocked by phentolamine, and partially blocked by propranolol. It is concluded that the perineurium possesses an octopamine receptor that could respond to octopamine circulating in the haemolymph, and which appears to mediate a reduction in the potassium sensitivity of the basolateral membrane of these glia.

INTRODUCTION

The blood–brain barrier of insects has been suggested by several investigations to be formed chiefly by the superficial layer of neuroglia of the central nervous system, the perineurium (see Treherne, 1974; Abbott & Treherne, 1977). This hypothesis has recently been confirmed for the perineurium of the cockroach by electrophysiological evidence (Schofield, Swales & Treherne, 1984*a*,*b*; Schofield & Treherne, 1984).

Hormonal control of the perineurium or other neuroglia in insects has never been studied. Neuroglia from other animals are known to be sensitive to a range of humoral factors, from experiments upon cultured cells (see Van Calker & Hamprecht, 1980) and a few *in situ* (see Villegas, 1981; Walz & Schlue, 1982; Reale, Evans & Villegas, 1986). There is some evidence to suggest that the function of

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perineurial cells is controlled by octopamine. The perineurial cells of the locust are known to contain an adenyl cyclase that is stimulated by 10^{-5} mol l^{-1} octopamine (Benedeczky & S-Rozsa, 1981) but the specificity of this effect is unknown. Also, at the abnormally high concentration of 10^{-4} mol l^{-1}, octopamine has been observed to stimulate the Na-dependent respiration of cockroach nerve cords: since this effect was blocked by an agent that lowers Na permeability, it was suggested that octopamine raises the Na permeability of the blood–brain barrier (Steele & Chan, 1980) although the site of action of octopamine was not identified.

In this study we have examined the sensitivity of the perineurial glia to octopamine by characterizing effects upon the trans-perineurial potential generated by these glia (Schofield & Treherne, 1984), and then examining the specificity and antagonism of the responses. A preliminary account of this work has been published (Schofield & Treherne, 1985).

METHODS

Electrophysiological recordings were made from the abdominal nerve cord of adult male cockroaches, *Periplaneta americana*, reared in laboratory culture. The cord was mounted in a Perspex chamber, with the connectives between the fifth and sixth ganglia under flowing saline. Recordings were made of the trans-perineurial potential in one of these connectives, relative to the bathing medium, using a microelectrode inserted into the sub-perineurial interstitial system, essentially as described earlier (Schofield *et al.* 1984*a*). Different salines were passed for periods that were measured with the aid of an electronic timer.

Saline was as used in a previous investigation (Schofield & Treherne, 1984), having a pH of 7·2 and the following composition (mmol l^{-1}): Na, 127; K, 3; Ca, 2; Mg, 2; mannitol, 50; trehalose, 5; Cl, 135; OH, 3; Hepes, 8·6. Low-Na saline (27 mmol l^{-1} Na) was made by substitution of 100 mmol l^{-1} Tris for 100 mmol l^{-1} Na (with a slight reduction in Cl content). Salines were filtered (0·45 μm Millipore) shortly before use. High-K saline (66·5 mmol l^{-1} K) was made by substitution of 63·5 mmol l^{-1} K for the equivalent amount of Na. In Tris-substituted saline, some Na was left to avoid any harmful effects of Na-free conditions (e.g. upon junctional permeability). In the high-K saline, K concentration was raised only as high as necessary to produce a response of useful size, again to avoid any harmful effects.

Glass microelectrodes were pulled from 1·0 mm o.d. thin-walled glass (Clark Electromedical), filled with filtered (0·2 μm Millipore) 3 mol l^{-1} KCl at pH 7·2 (3 mmol l^{-1} Hepes), and had a resistance of 4–8 MΩ.

Most pharmacological agents were supplied by Sigma, with the exception of DL-adrenaline HCl, which was obtained from Pfaltz & Bauer, Inc., and phentolamine mesylate which was kindly donated by Ciba Geigy Pharmaceuticals. All solutions were used within 8 h of preparation.

Values are given as median (mean ± S.E.M.). N = no. of observations = no. of preparations. Statistical analysis of differences was made using the Mann–Whitney U-test, and probability (P) was considered significant with a value less than 0·05.

RESULTS

Effect of octopamine upon trans-perineurial potential

The hypothesis that external application of 10^{-4} mol l^{-1} octopamine to the cockroach nervous system lowers the Na permeability of the blood–brain barrier (Steele & Chan, 1980) was tested by examining the effect of such exposure upon the interstitially recorded trans-perineurial potential (V_s). Changes in the p.d. (dV_s) were evoked by exposure to low-Na (Tris) saline for 30 s at 5-min intervals, to give a measure of relative Na permeability (Pichon, Moreton & Treherne, 1971). In control experiments, in the absence of octopamine, there was a gradual fall in V_s and a gradual increase in the amplitude of the Tris-induced dV_s. A standard procedure for experiments was therefore adopted in which cockroaches were obtained fresh from the culture, and impalements were made within 10–20 min after setting up the preparation. Decline in V_s and increase in dV_s were steady by 45 min after setting up the preparation. Octopamine was applied at 60 min after set-up in six preparations, when the median value of V_s was 17·0 mV (16·1 ± 1·86), not significantly different from the level in controls ($N = 7$), 15·9 mV (15·2 ± 1·81). At 5 min before octopamine, dV_s was 0·3 mV (0·5 ± 0·23), not significantly different from the effect in controls at the comparable time (55 min), 0·2 mV (0·04 ± 0·20).

Upon application of 10^{-4} mol l^{-1} DL-octopamine, there was a positive shift in the interstitial potential over a period of about 5 min, which was sometimes accompanied by an increase in the amplitude of Tris-induced changes (Fig. 1). Only the increase in V_s was significant: V_s increased by 2·5 mV (3·6 ± 1·62) during the first 5 min of octopamine, significantly greater ($P < 0·01$) than in controls between 60 and 65 min,

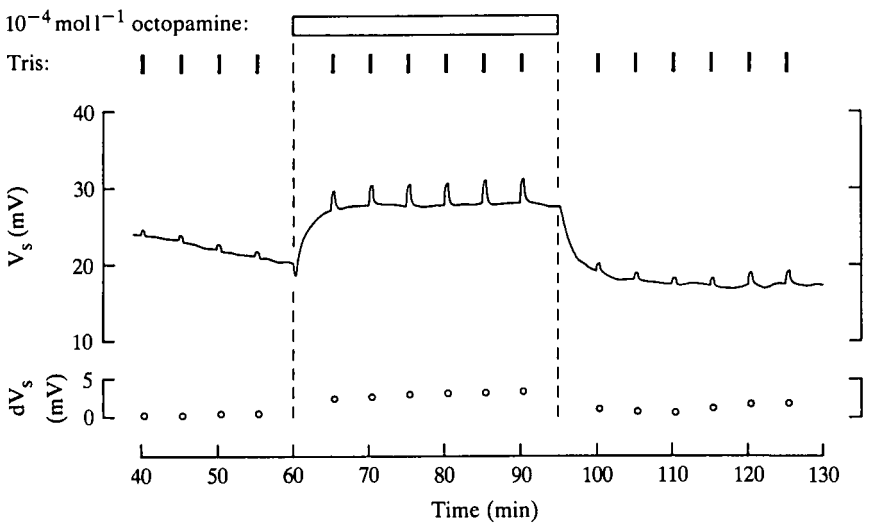

Fig. 1. Effect of 10^{-4} mol l^{-1} DL-octopamine upon interstitial potential (V_s) in a cockroach central nervous connective and upon the changes in potential (dV_s) induced by 30-s pulses of 100 mmol l^{-1} Tris. Top trace shows continuous recording from the microelectrode while the graph shows the amplitude of dV_s, measured at the end of each Tris pulse. Time scale shows elapsed time from setting up the preparation.

$-0{\cdot}9$ mV ($-0{\cdot}8 \pm 0{\cdot}096$); while dV_s increased by $0{\cdot}6$ mV ($1{\cdot}0 \pm 0{\cdot}42$) during 30 min octopamine, no different to the change in controls over the comparable period, $0{\cdot}4$ mV ($0{\cdot}5 \pm 0{\cdot}12$).

The effect upon V_s of 30 min exposure to octopamine was reversible (Fig. 1).

Effect of octopamine upon K-induced changes in potential

To provide further information about the effects of octopamine, preparations were exposed at 5-min intervals to a 15-s pulse of 67 mmol l^{-1} K, 45 s after a 15-s pulse of Tris. Pulses of high K have been used earlier to characterize the barrier (see Schofield *et al.* 1984*a*,*b*; Schofield & Treherne, 1984). In control experiments, V_s showed a similar time course to that in the preceding section, while K-induced dV_s declined slowly. This decline was steady after about 45 min. Before octopamine ($N = 7$), values of V_s at 60 min, $12{\cdot}2$ mV ($11{\cdot}1 \pm 1{\cdot}41$), Tris-induced dV_s at 55 min, $0{\cdot}4$ mV ($0{\cdot}3 \pm 0{\cdot}10$) and K-induced dV_s at 56 min, $32{\cdot}7$ mV ($32{\cdot}7 \pm 1{\cdot}84$) were not

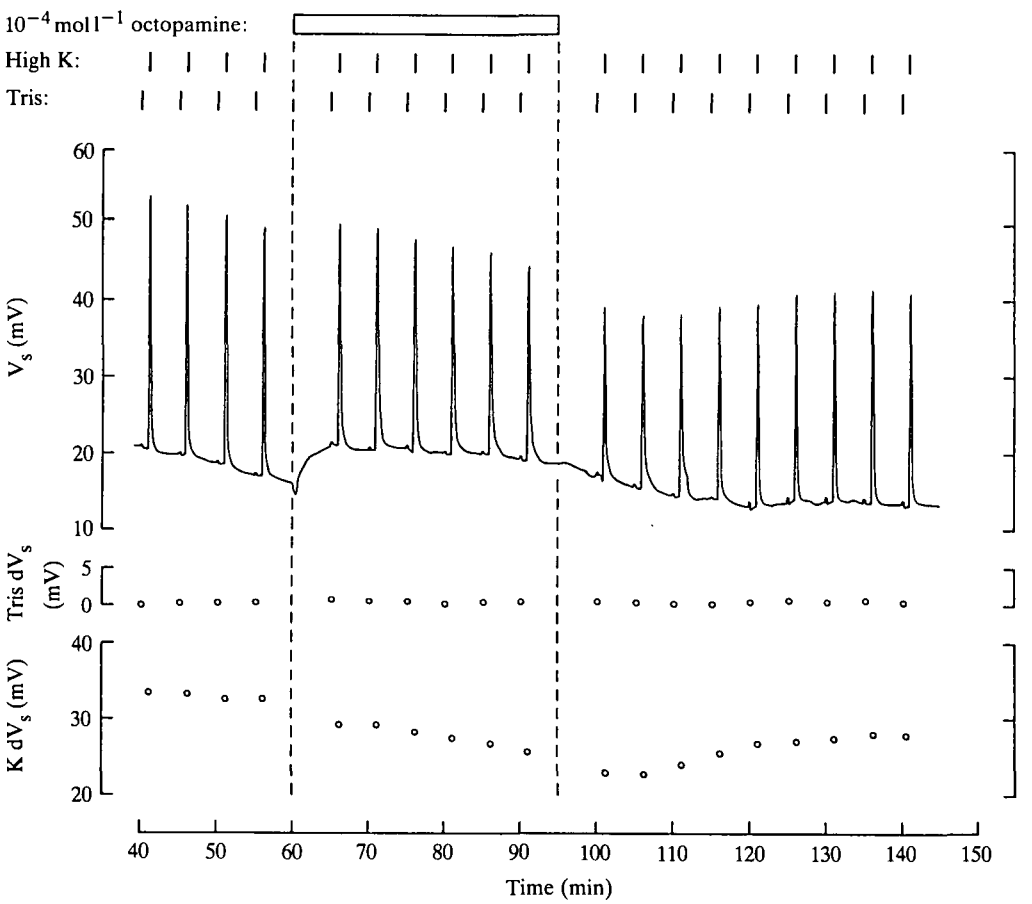

Fig. 2. Effect of 10^{-4} mol l^{-1} DL-octopamine upon interstitial potential (V_s) and upon dV_s induced by pulses of 100 mmol l^{-1} Tris for 15 s, and of 67 mmol l^{-1} K for 15 s. Display as in Fig. 1.

significantly different from values at comparable times in controls, which showed a V_s of 10·7 mV (10·8 ± 1·71) ($N = 7$), a Tris-induced dV_s of 0·4 mV (0·4 ± 0·072) ($N = 8$) and a K-induced dV_s of 32·5 mV (33·6 ± 2·31) ($N = 8$).

Treatment with 10^{-4} mol l^{-1} octopamine was found to raise the level of V_s with no effect upon Tris-induced dV_s, as in the preceding experiments, while a progressive reduction in the amplitude of the K-induced dV_s was observed (Fig. 2). V_s increased over 5 min by 1·9 mV (2·0 ± 0·59), more ($P < 0·01$) than the change in controls, −0·6 mV (−0·5 ± 0·23). Tris-induced dV_s changed after 30 min in octopamine by 0·2 mV (0·2 ± 0·13), no different to that in controls, 0·1 mV (0·2 ± 0·074). After 31 min in octopamine, K-induced dV_s was reduced by 6·9 mV (−7·0 ± 1·3), significantly different ($P < 0·05$) from the reduction of −1·5 mV (−1·7 ± 0·41) observed in controls over a comparable period.

The rise in V_s was generally less than the fall in dV_s, but sometimes the rise was more than adequate to account for the fall, such that the value of V_s in high K was carried to a higher absolute level than before octopamine (e.g. Fig. 2).

After 30 min exposure to octopamine, the effects appeared to be reversible (Fig. 2) compared to observations in controls. Recovery was occasionally preceded by a temporary reduction in dV_s (Fig. 2), again suggesting that more than one mechanism was involved.

Concentration dependence of octopamine effects

The effects of DL-octopamine upon the interstitial potential and K-induced steps were then examined for a range of octopamine concentrations from 10^{-8} mol l^{-1} to 10^{-4} mol l^{-1}, employing 15-s pulses of 67 mmol l^{-1} K at 5-min intervals, and octopamine application at 60 min after set-up. A pulse length of 15 s was adopted because the effects produced were nearly as great as those produced by 30-s pulses. Values of V_s and dV_s before the test exposure were not significantly different from values in controls for these experiments (Table 1) and those in subsequent sections (Tables 2–4).

The effect upon the resting level of V_s was significant down to a concentration of 5×10^{-7} mol l^{-1}: during the first 5 min of 5×10^{-7} mol l^{-1} octopamine application, V_s rose by 1·0 mV, compared to a value of −0·5 mV in controls (Table 1). An effect upon the K-induced dV_s was found after only 5 min exposure to octopamine at concentrations down to 10^{-7} mol l^{-1} (Fig. 3). At this concentration, the effect appeared complete after 10 min, and a dose–response curve was prepared for the effect upon K-induced dV_s after 10 min octopamine (Fig. 4). At 10^{-5} mol l^{-1} and 10^{-4} mol l^{-1}, there was a progressive decline in dV_s during the exposure, and hysteresis afterwards, similar to that shown in the preceding section for 10^{-4} mol l^{-1} (Fig. 2).

Amine specificity of the effect upon K-induced dV_s

To examine the specificity of the response to octopamine, a range of biogenic amines was tested for similar effects. Since a marked reduction in the amplitude of K-induced steps in potential was found after 10 min exposure to octopamine at

Table 1. *Comparison of values of interstitial potential (V_s) and K-induced steps (dV_s) over a range of concentrations of* DL-*octopamine with those in controls*

	V_s at 60 min	Change in V_s at 65 min	dV_s at 55 min	Change in dV_s at 70 min
Controls ($N=8$)	12·5 (11·0 ± 3·57)	−0·5 (−0·3 ± 0·25)	31·2 (28·8 ± 2·57)	−0·3 (−0·5 ± 0·23)
10^{-8} mol l^{-1} (V_s, $N=6$; dV_s, $N=7$)	13·8 (13·5 ± 1·98) NS	−0·3 (−0·1 ± 0·45) NS	25·3 (25·8 ± 1·94) NS	−2·0 (−1·4 ± 0·79) NS
10^{-7} mol l^{-1} ($N=7$)	10·9 (10·7 ± 1·36) NS	0·5 (0·4 ± 0·19) NS	28·8 (28·7 ± 2·56) NS	−5·7 (−6·2 ± 1·07) $P<0·002$
5×10^{-7} mol l^{-1} ($N=10$)	17·5 (15·1 ± 2·23) NS	1·5 (1·7 ± 0·54) $P<0·02$	29·7 (28·2 ± 2·0) NS	−5·9 (−6·0 ± 0·59) $P<0·002$
10^{-6} mol l^{-1} ($N=7$)	13·6 (13·4 ± 0·95) NS	1·6 (1·5 ± 0·18) $P<0·002$	31·6 (29·7 ± 2·57) NS	−7·9 (−7·7 ± 1·72) $P<0·05$
10^{-5} mol l^{-1} ($N=7$)	14·6 (14·9 ± 1·42) NS	1·7 (3·0 ± 1·10) $P<0·01$	30·8 (31·3 ± 2·18) NS	−4·2 (−4·7 ± 0·63) $P<0·002$
10^{-4} mol l^{-1} ($N=7$)	14·7 (13·4 ± 2·12) NS	4·4 (4·0 ± 0·98) $P<0·05$	27·4 (28·9 ± 1·71) NS	−7·9 (−7·5 ± 0·61) $P<0·002$

Pulses of 67 mmol l^{-1} K for 15 s were given at 5-min intervals, and octopamine was applied at 60 min after set-up, as in Fig. 3.

Values (in mV) expressed as: median (mean ± S.E.M.).

concentrations as low as 10^{-7} mol l^{-1} the amines were tested at this concentration using the same protocol as in the preceding section, comparing with the same controls (Table 2).

Of the amines tested, none had a significant effect upon the resting level of V_s, but several affected the K-induced dV_s (Table 2).

Only DL-synephrine was like octopamine in causing a reduction of dV_s, but the effect was smaller, with a reduction of 4·1 mV compared to 5·7 mV during octopamine. The other phenolamine tested, tyramine, had no significant effect upon dV_s.

Among the catecholamines, an increase in dV_s was produced upon β-hydroxylation, and *N*-methylation appeared to enhance the effect: dopamine had no effect, DL-noradrenaline produced a slight increase of 0·5 mV and DL-adrenaline gave an increase of 1·2 mV.

With the three other compounds related to octopamine, phenylethylamine produced an increase in dV_s of 1·1 mV, DL-phenylethanolamine had no significant effect, but DL-*N*-methylphenylethanolamine caused a significant increase of 0·6 mV.

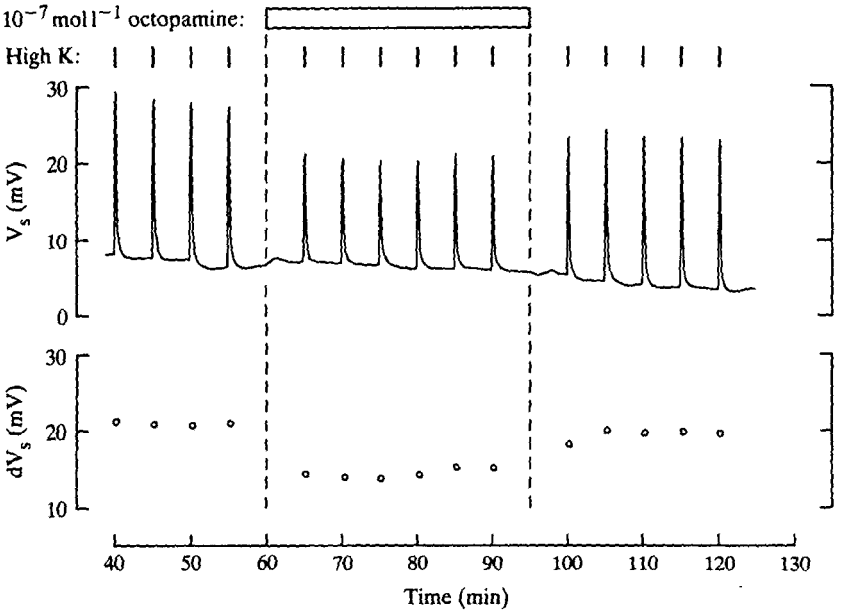

Fig. 3. Effect of 10^{-7} mol l^{-1} DL-octopamine upon interstitial potential (V_s) and upon dV_s induced by pulses of 67 mmol l^{-1} K for 15 s. Display as in Fig. 1.

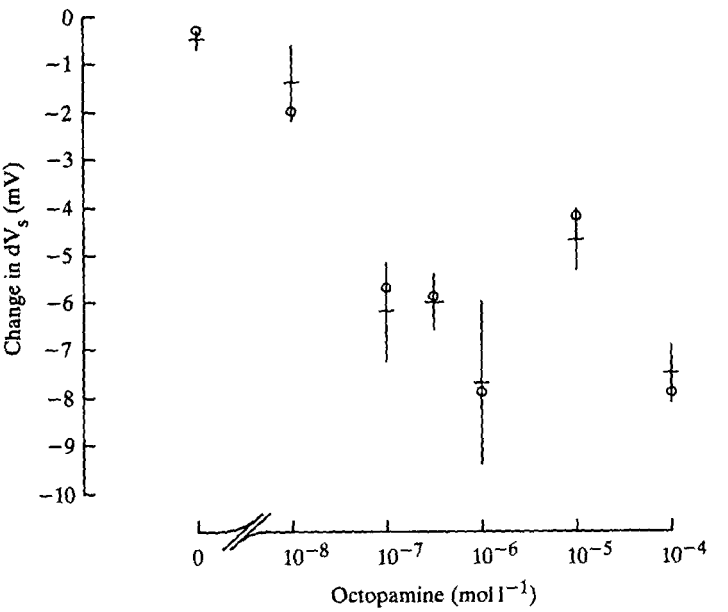

Fig. 4. Dose–response curve for effect of 10-min exposure to DL-octopamine upon K-induced steps in interstitial potential (dV_s), in experiments as in Fig. 3. The change in dV_s between the value obtained at 55 min after set-up (5 min before octopamine) and that obtained at 70 min after set-up (10 min octopamine) (Table 1) is plotted as median (○) and mean (horizontal bar) ± S.E.M. (vertical bar).

Table 2. *Comparison between values of interstitial potential (V_s) and K-induced steps (dV_s) in preparations exposed to various amines at 10^{-7} mol l^{-1} with those in controls*

	V_s at 60 min	Change in V_s at 65 min	dV_s at 55 min	Change in dV_s at 70 min
Controls ($N = 8$)	12·5 (11·0 ± 3·57)	−0·5 (−0·3 ± 0·25)	31·2 (28·8 ± 2·57)	−0·3 (−0·5 ± 0·23)
Glycine ($N = 4$)	19·9 (19·1 ± 3·60) NS	−0·3 (−0·1 ± 0·60) NS	28·1 (30·0 ± 2·33) NS	−0·5 (−0·8 ± 1·29) NS
Glutamate (V_s, $N = 6$; dV_s, $N = 7$)	13·5 (12·7 ± 1·59) NS	−0·3 (−0·3 ± 0·21) NS	32·3 (29·4 ± 3·45) NS	−0·4 (−0·4 ± 0·41) NS
γ-Aminobutyric acid ($N = 6$)	11·7 (11·5 ± 2·24) NS	−0·6 (−0·7 ± 0·31) NS	29·3 (30·5 ± 3·10) NS	−1·7 (−1·6 ± 0·43) NS
Carbamylcholine ($N = 6$)	13·1 (12·9 ± 2·25) NS	−0·5 (−0·48 ± 0·18) NS	33·4 (31·4 ± 2·10) NS	0·5 (0·5 ± 0·23) $P < 0·05$
Histamine ($N = 5$)	11·3 (11·8 ± 1·93) NS	−0·4 (−0·4 ± 0·14) NS	30·1 (31·3 ± 2·88) NS	0·1 (0·1 ± 0·33) $P < 0·05$
5-Hydroxytryptamine ($N = 5$)	5·8 (6·9 ± 2·03) NS	0·0 (0·4 ± 0·34) NS	32·3 (30·8 ± 2·53) NS	−0·4 (−0·9 ± 0·50) NS
p-Tyramine ($N = 8$)	17·9 (17·2 ± 1·65) NS	0·2 (0·1 ± 0·46) NS	31·1 (29·2 ± 2·92) NS	−0·8 (−1·2 ± 0·50) NS
DL-*p*-octopamine ($N = 7$)	10·9 (10·7 ± 1·36) NS	0·5 (0·4 ± 0·19) NS	28·8 (28·7 ± 2·56) NS	−5·7 (−6·2 ± 1·07) $P < 0·002$
DL-*p*-synephrine ($N = 5$)	14·7 (12·7 ± 2·89) NS	0·5 (0·5 ± 0·15) NS	30·6 (31·5 ± 1·61) NS	−4·1 (−4·3 ± 0·73) $P < 0·01$
Phenylethylamine ($N = 6$)	15·9 (15·5 ± 1·82) NS	−0·6 (−0·5 ± 0·22) NS	30·3 (29·6 ± 2·14) NS	1·1 (1·1 ± 0·34) $P < 0·02$
DL-phenylethanolamine ($N = 6$)	12·0 (11·4 ± 2·41) NS	−0·6 (−0·7 ± 0·21) NS	32·6 (31·6 ± 2·7) NS	0·1 (0·2 ± 0·29) NS
DL-*N*-methylphenyl-ethanolamine ($N = 6$)	13·0 (10·6 ± 2·58) NS	0·0 (0·2 ± 0·33) NS	32·3 (31·8 ± 2·39) NS	0·6 (0·6 ± 0·19) $P < 0·002$
Dopamine ($N = 6$)	14·1 (13·2 ± 1·50) NS	−0·5 (−0·5 ± 0·14) NS	33·1 (31·1 ± 1·93) NS	−0·3 (−0·5 ± 0·28) NS
DL-noradrenaline ($N = 6$)	17·9 (17·2 ± 0·91) NS	−0·6 (−0·7 ± 0·16) NS	29·8 (31·1 ± 2·25) NS	0·5 (0·5 ± 0·28) $P < 0·05$
DL-adrenaline ($N = 6$)	15·0 (16·5 ± 2·65) NS	−0·2 (−0·4 ± 0·29) NS	20·6 (27·1 ± 2·10) NS	1·2 (1·1 ± 0·50) $P < 0·05$

Pulses of 67 mmol l^{-1} K were applied at 5-min intervals, and octopamine was applied at 60 min after set-up, as in Fig. 4.

Values (in mV) expressed as: median (mean ± S.E.M.).

Glycine, glutamate, γ-aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT) had no significant effect upon dV_s, whereas histamine and carbamylcholine induced a slight but significant increase in dV_s.

The effect of 10^{-7} mol l^{-1} DL-octopamine, to cause a fall in the size of the K-induced dV_s in the absence of any effect upon resting V_s, thus appears to be very specific and is mimicked, only partially, by the *N*-methylated derivative, synephrine.

Antagonists of octopamine action

To provide some characterization of the receptor upon which octopamine was acting, a comparison was made between the effects of phentolamine, an antagonist

Table 3. *Comparison between values of interstitial potential (V_s) and K-induced steps (dV_s) in preparations exposed to adrenergic blockers at 10^{-6} mol l^{-1} with those in controls*

	V_s at 45 min	Change in V_s at 50 min	dV_s at 40 min	Change in dV_s at 55 min
Controls (V_s, $N=7$; dV_s, $N=8$)	13·6 (15·1 ± 1·82)	−0·7 (−0·6 ± 0·42)	32·4 (29·8 ± 2·45)	−1·2 (−1·0 ± 0·29)
Phentolamine ($N=9$)	17·9 (17·3 ± 6·23) NS	−0·7 (−0·7 ± 0·22) NS	30·2 (29·5 ± 2·78) NS	0·0 (0·1 ± 0·25) $P<0·05$
DL-propranolol ($N=8$)	16·9 (17·0 ± 1·16) NS	0·0 (−0·1 ± 0·17) NS	31·3 (31·1 ± 1·41) NS	0·0 (0·2 ± 0·39) $P<0·05$

Pulses of 67 mmol l^{-1} K were applied at 5-min intervals, and blockers were applied at 45 min after set-up, as in Fig. 5.
Values (in mV) expressed as: median (mean ± S.E.M.).

Table 4. *Comparison between values of interstitial potential (V_s) and K-induced steps (dV_s) in preparations exposed to octopamine at 10^{-7} mol l^{-1} in the presence of adrenergic blockers at 10^{-6} mol l^{-1} with those in controls*

	V_s at 60 min	Change in V_s at 65 min	dV_s at 55 min	Change in dV_s at 70 min
Controls ($N=8$)	12·5 (11·0 ± 3·57)	−0·5 (−0·3 ± 0·25)	31·2 (28·8 ± 2·57)	−0·3 (−0·5 ± 0·23)
Octopamine and phentolamine ($N=9$)	15·5 (15·3 ± 2·04) NS	−0·1 (−0·2 ± 0·16) NS	30·7 (29·6 ± 2·91) NS	−1·9 (−1·4 ± 0·39) NS
Octopamine and DL-propranolol ($N=9$)	14·5 (15·1 ± 1·19) NS	1·4 (1·8 ± 0·39) $P<0·002$	33·0 (31·7 ± 1·31) NS	−2·7 (−3·3 ± 0·73) $P<0·002$

Pulses of 67 mmol l^{-1} K were applied at 5-min intervals, blockers were applied at 45 min after set-up, and octopamine was applied at 60 min, as in Fig. 5.
Values (in mV) expressed as: median (mean ± S.E.M.).

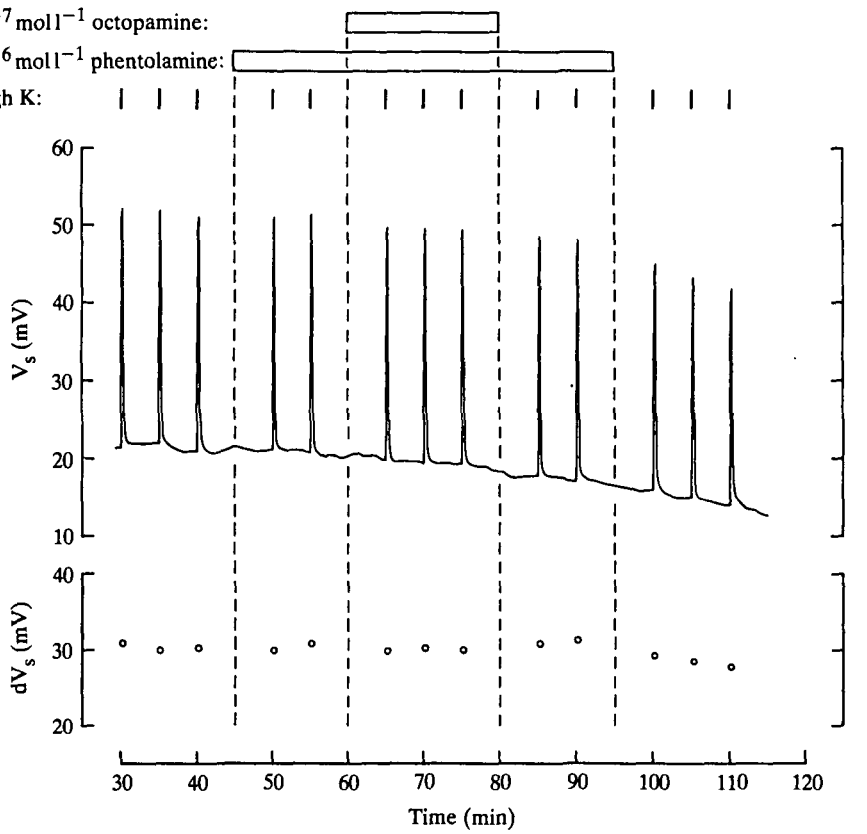

Fig. 5. Exposure to 10^{-6} mol l^{-1} phentolamine before and during application of 10^{-7} mol l^{-1} DL-octopamine. Display as in Fig. 3. Phentolamine had no effect upon interstitial potential (V_s) but halted the decline in K-induced dV_s (see Table 3). During exposure to this α-adrenergic blocker, octopamine has no effect upon dV_s (see Table 4).

of α-adrenergic receptors, and propranolol, a β-blocker, upon the response to octopamine. Preparations were exposed to the agents at a concentration of 10^{-6} mol l^{-1} for 15 min prior to, and during, exposure to 10^{-7} mol l^{-1} DL-octopamine. Octopamine was applied at the standard time of 60 min after set-up. Effects were compared with those in controls at comparable times (Tables 3, 4).

Upon application of phentolamine (Fig. 5) or DL-propranolol (Fig. 6), the value of dV_s ceased declining, but there was no effect upon resting V_s (Table 3).

In the presence of phentolamine, octopamine had no effect upon either V_s or dV_s (Fig. 5; Table 4). This contrasts with the reduction in dV_s observed in octopamine in the absence of phentolamine (Table 1).

In contrast, in the presence of 10^{-6} mol l^{-1} DL-propranolol, 10^{-7} mol l^{-1} octopamine increased V_s by 1·4 mV, significantly different from the effect in controls, and depressed K-induced dV_s by 2·7 mV (Fig. 6; Table 4). Thus there was a greater effect upon V_s and less effect upon dV_s than in 10^{-7} mol l^{-1} octopamine alone (Table 1).

DISCUSSION

The glia which form the blood–brain barrier of the cockroach showed a response to octopamine that was unlike that given to a range of other biogenic amines. At a concentration of 10^{-7} mol l^{-1}, the response was revealed as a reduction in the potassium sensitivity of the potential that is generated across the perineurium. Only synephrine produced a similar response, but was less effective. In invertebrates, several octopamine-sensitive systems also show a response to synephrine. The relative potency observed in the present system is consistent with that obtained for homogenates of whole cockroach brain (Harmar & Horn, 1979), and is also found for the induction of hypertrehalosaemia in cockroach haemolymph (Downer, 1979). Synephrine is more potent than octopamine in the threshold for effects in the locust upon myogenic rhythm (Evans & O'Shea, 1978) and muscle twitch tension (O'Shea & Evans, 1979). Some systems show equal sensitivities, such as locust neurosecretory cells (Orchard, Gole & Downer, 1983). A further similarity of the effect upon the perineurium with effects mediated by octopamine receptors in other

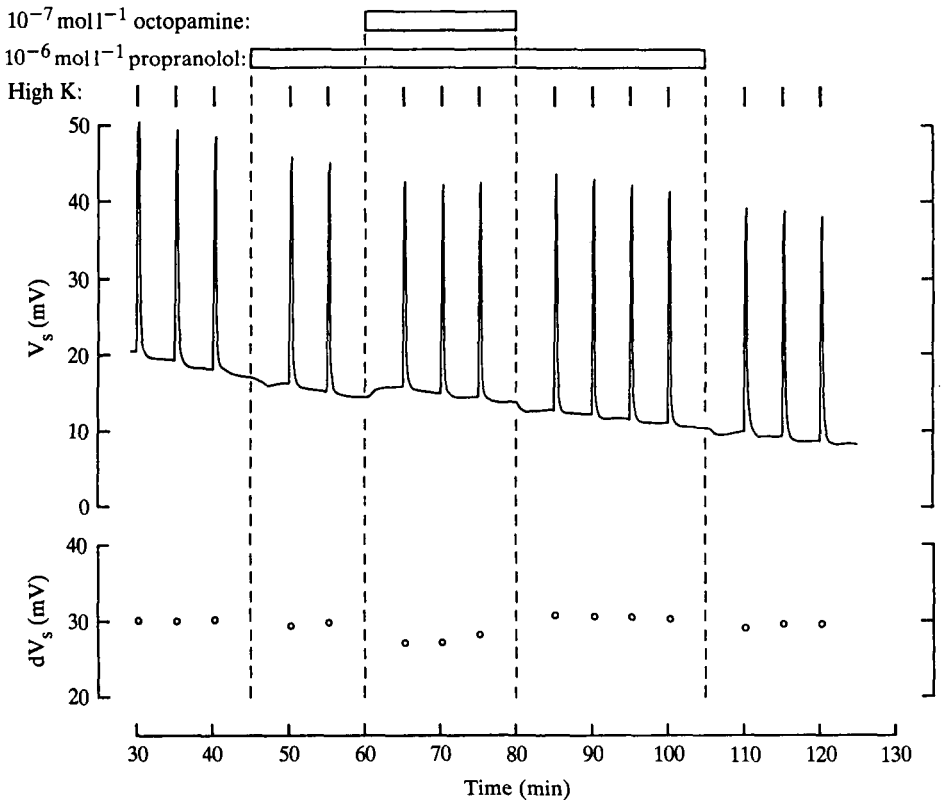

Fig. 6. Exposure to 10^{-6} mol l^{-1} DL-propranolol before and during application of 10^{-7} mol l^{-1} DL-octopamine. Display as in Fig. 3. Propranolol had no effect upon interstitial potential (V_s) but halted the decline in K-induced dV_s (see Table 3). During exposure to this β-adrenergic blocker, octopamine induced a positive shift in V_s and there was a partial inhibition of the effect of octopamine upon dV_s (see Table 4).

invertebrates is the exhibition of a more effective blockage by phentolamine than by propranolol (see Evans, 1981). Such antagonism is shown by α-adrenergic receptors, but the substantial block by propranolol may indicate the presence of receptors with β-adrenergic characteristics. It should also be noted that an effect of octopamine upon the resting level of the interstitial potential was revealed in the presence of propranolol.

Most of the known octopamine receptors show a sensitivity to catecholamines and phenylamines as well as to phenolamines (see Harmar, 1980). In the present study, the only effect observed for such compounds was an increase in potassium sensitivity, whereas a decrease was observed with octopamine. Among these compounds, DL-adrenaline appeared most potent. This suggests that there is an adrenergic receptor upon the perineurium. Adrenergic receptors are found upon cultured vertebrate glia (Van Calker & Hamprecht, 1980). Insects have not been demonstrated to possess adrenergic receptors (see Brown & Nestler, 1985) or receptors for phenylamines (see Leake & Walker, 1980), but the nervous system may contain traces of adrenaline and noradrenaline (see Pitman, 1985).

The perineurium might also possess histaminergic and cholinergic receptors that mediate an increase in K sensitivity, since the K-induced potentials were enhanced by histamine and by carbachol. Cholinergic receptors have been found in squid glia (see Villegas, 1981) while receptors for both compounds are known in cultured vertebrate glia (see Van Calker & Hamprecht, 1980). The perineurium may therefore be the site of some of those adenylate cyclases in the insect nervous system that have been demonstrated to be sensitive to histamine (see Brown & Nestler, 1985) and acetylcholine (see Sattelle, 1985). Eserine-sensitive acetylcholinesterase activity has been detected in cockroach glial cells (Smith & Treherne, 1965). Also, binding of α-bungarotoxin has been observed in superficial regions of cockroach ganglia, but further investigation is required to see if this could include binding to the perineurium (Sattelle *et al.* 1983).

The observed effects of octopamine upon the perineurium indicate an action upon at least one of the electrical parameters that contribute to the trans-perineurial potential (Fig. 7; see also Schofield & Treherne, 1984): namely the e.m.f.s due to the paracellular shunt (E_s), basolateral membrane (E_b) and apical membrane (E_a), and the resistances due to these structures (R_s, R_b and R_a, respectively). E_s is likely to be small and have little influence (Schofield & Treherne, 1984). The potassium-induced positive steps in the potential (dV_s) may be interpreted as due to the depolarization of the basolateral e.m.f., together with some reduction in basolateral resistance. A reduction in dV_s, as observed at octopamine concentrations as low as 10^{-7} mol l^{-1}, can be seen in terms of the model to be simulated by certain effects upon the shunt or membranes (Fig. 8). Thus such an effect could be caused by a decrease in R_s (Fig. 8A) but would then be accompanied by reductions in V_s and R_t, which have not been seen: no significant effect upon V_s was observed at this concentration, and R_t has been found to rise during such treatment (Schofield & Treherne, 1985). A reduction in dV_s could also be caused by a higher resistance of the basolateral membrane (Fig. 8B), but the effect upon the resting level of V_s would

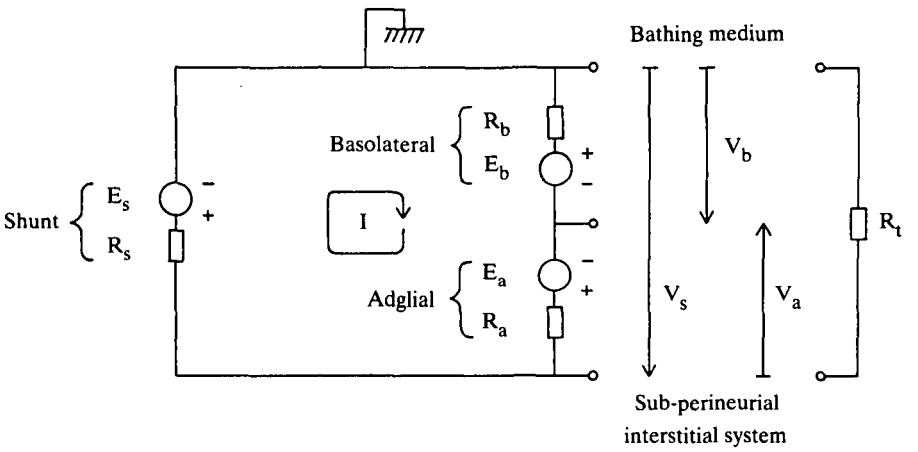

Fig. 7. Electrical model in which the perineurium is represented by sources of e.m.f. (E) and by resistances (R), and has an equivalent resistance (R_t). The model consists of a paracellular shunt (E_s, R_s), the basolateral membrane of the cell (E_b, R_b) which faces the bathing medium and the adglial membrane (E_a, R_a) which faces the underlying glia. A current (I) flows through the circuit so that a change in any parameter of the model can affect the voltage recorded across the shunt (V_s), basolateral membrane (V_b) and adglial membrane (V_a). V_s is the voltage recorded in the interstitial system across the perineurium. After Schofield & Treherne (1984).

be the opposite to that observed. However, if the resistance of this membrane rose because the potassium conductance fell, there would be an accompanying reduction in e.m.f. Such a combined effect could produce a decrease in dV_s with a small increase in both V_s and R_t (Fig. 8C), as seen. A rise in resistance of the adglial membrane would have much less effect (Fig. 8D) than an increase in R_b, while a rise in R_a and fall in E_a together would produce a small decrease in dV_s that would be accompanied by a large drop in V_s (Fig. 8E). Thus the decrease in K-induced dV_s produced by low concentrations of octopamine is best explained by a reduction in the potassium sensitivity of the basolateral membrane: at external concentrations of K below the perineurial intracellular concentration, the reduction in e.m.f. would give rise to a positive shift in trans-perineurial potential that would be countered by a negative shift due to the higher membrane resistance; at high K concentrations, both the decrease in potassium sensitivity and the higher resistance would depress the positive shift in trans-perineurial potential.

An effect of octopamine upon the basolateral membrane could easily be accompanied by an effect upon some other parameter of the perineurium. At high octopamine concentrations, the increase in the resting level of the trans-perineurial potential and the suggestion of more than one mechanism might reflect a reduction in basolateral e.m.f. becoming more important relative to the effect of a rise in R_b, but these data could also reflect an increase in the value of R_s during the octopamine exposure.

Octopamine has been shown in preliminary experiments to cause a reduction in the net K permeability of the perineurium (Schofield & Treherne, 1985). Such an

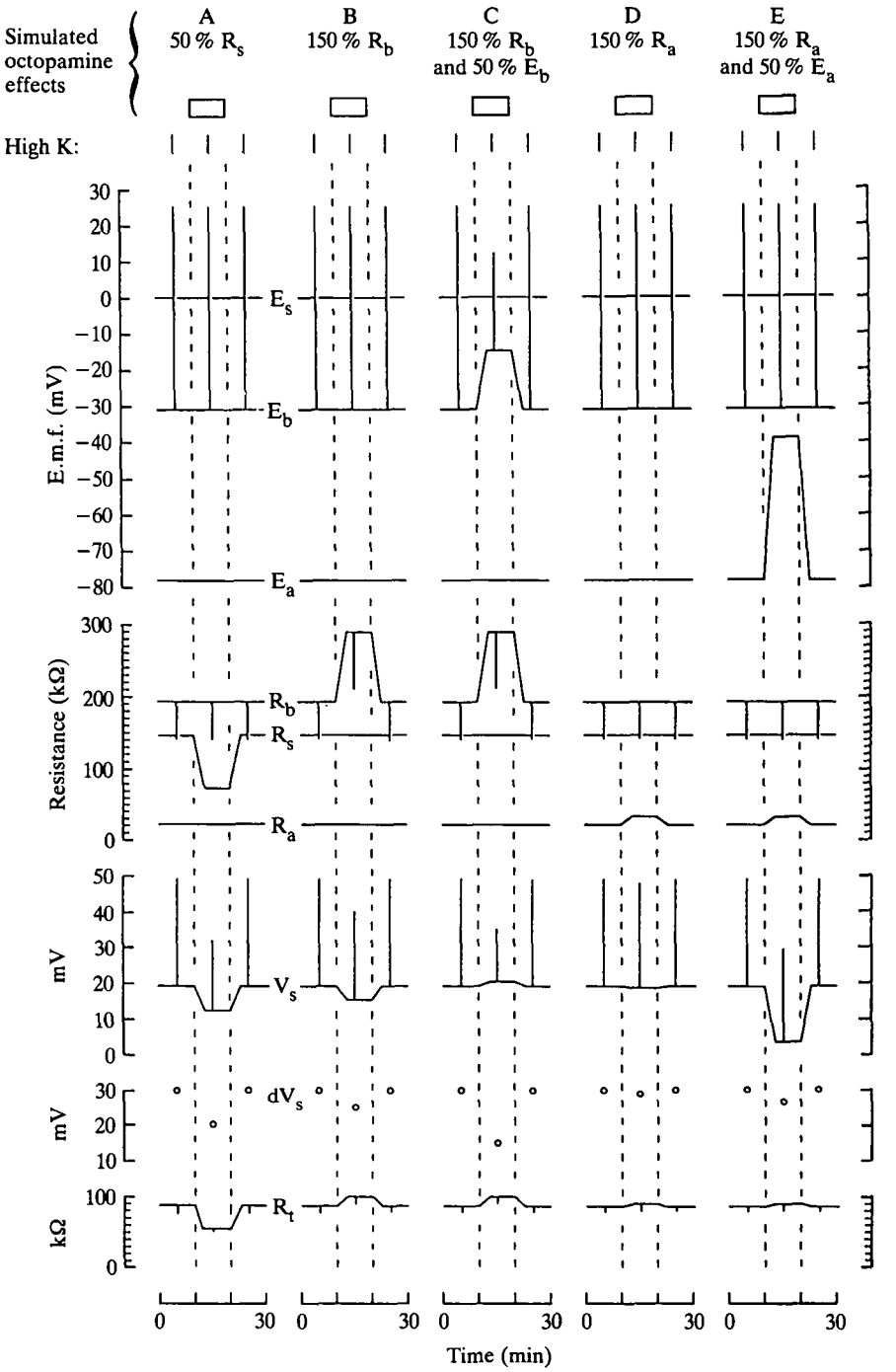

Fig. 8. Simulation of how octopamine could reduce the amplitude of potassium-induced changes in interstitial potential (dV_s) by effects upon parameters of the electrical model of the perineurium (Fig. 7), using values of e.m.f. (E) and resistance (R) obtained by Schofield & Treherne (1984) in normal and high K (130 mmol l^{-1}). Values of the interstitial potential (V_s), potassium-induced changes in potential (dV_s) and resistance across the perineurium (R_t) are calculated for values of e.m.f. and resistance employing the equations of Schofield & Treherne (1984). Simulations are made of possible effects of octopamine: (A) to reduce R_s by 50 %, (B) to raise R_b by 50 %, (C) to raise R_b by 50 % with a 50 % reduction in E_b, (D) to raise R_a by 50 % and (E) to raise R_a by 50 % with a 50 % reduction in E_a. These effects are simulated to be complete within 3 min, and reverse in 3 min.

effect could be caused by either of the above possibilities: by a fall in K permeability of the basolateral membrane or by a rise in intercellular resistance.

Octopamine, at the high concentration of 10^{-4} mol l^{-1}, did not appear to increase the sodium permeability of the perineurium as has been proposed by Steele & Chan (1980). If octopamine raised Na permeability selectively, the Tris-induced changes in potential could be expected to become more negative (Schofield & Treherne, 1984). No such effect was observed.

Sensitivity of glial cells to octopamine has been observed in only one other instance, for the Schwann cells of the squid giant axon (Reale *et al.* 1986), where the membrane is hyperpolarized at low external potassium concentrations. In the present experiments, the basolateral membrane of the perineurium was indicated to depolarize under such conditions. A further major difference between the two systems is that tyramine is an effective agonist in the Schwann cell but not in the cockroach perineurium.

It is not known whether the glial cells of the squid are exposed to octopamine *in vivo*. In the cockroach, the levels of octopamine that are attained in the haemolymph following activity (6×10^{-8} mol l^{-1}) and stress (9×10^{-8} mol l^{-1}) (Davenport & Evans, 1984) are indicated by the present results to be sufficient to stimulate the perineurium. Alternatively, the perineurium might respond to octopamine release from glia, or from that contained in haemocytes (Davenport & Evans, 1984) or neurones (see Evans, 1985). Some elements of the octopamine uptake mechanisms demonstrated by Evans (1978) may be involved in a physiological response to octopamine.

The action of octopamine upon the perineurium might involve the stimulation of adenyl cyclase, since such activation has been observed at an octopamine concentration of 10^{-5} mol l^{-1} in the locust perineurium (Benedeczky & S-Rozsa, 1981). Such stimulation is obtained in a variety of systems (see Evans, 1985) including the squid Schwann cell (Reale *et al.* 1986). Elevated levels of cyclic AMP are known to raise the resistance of intercellular occlusions (Duffey, Hainau, Ho & Bentzel, 1981), which may be involved in the present responses, and can be expected to have other important effects upon the glia which form the insect blood–brain barrier.

Further investigations are being made into the nature of the effects of octopamine upon the perineurium, whether the effect is sensitive to formamidines, and the role of other humoral factors.

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