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Pharmacological characterization of the ergot alkaloid receptor in the salivary gland of the ixodid tick *Amblyomma hebraeum*

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Summary

Female ticks of the family Ixodidae osmoregulate by secreting the excess fluid of the blood meal back into the host's circulation *via* the salivary glands. At least three receptors control salivary fluid secretion in the tick *Amblyomma hebraeum*: (1) dopamine (DA) stimulates fluid secretion *via* a DA receptor, (2) ergot alkaloids (ErAs) stimulate fluid secretion *via* an ErA-sensitive receptor (the natural ligand of which has not been identified), and (3) a GABA receptor potentiates the action of DA and ErAs. Here we present some pharmacological properties of the ErA-sensitive receptor. Of the 11 ErAs we tested, (i) four were complete agonists (approximate concentration eliciting 50% maximum response is given in parentheses): dihydroergotamine (0.02 μmol l^{-1}), ergonovine (ErN; 0.06 μmol l^{-1}), methylergonovine (0.1 μmol l^{-1}) and α-ergocriptine (0.9 μmol l^{-1}); (ii) three were 'incomplete agonists' (approximate concentration eliciting 20% maximum response is given in parentheses): ergocorninine (3.5 μmol l^{-1}), ergocristinine (7.5 μmol l^{-1}) and ergocristine (10 μmol l^{-1}); (C) three were partial agonists (approximate concentration eliciting the respective maximum response in parentheses): ergocornine (50% maximum by 1 μmol l^{-1}), methysergide (28% maximum by 10 μmol l^{-1}) and bromocriptine (22% maximum by 10 μmol l^{-1}); and (D) one had no activity up to 1 mmol l^{-1}: ergothioneine. Bromocriptine and methysergide did not antagonize the action of DA, but were effective competitive antagonists of ErN, with K_is of ~0.3 μmol l^{-1} and 11 μmol l^{-1}, respectively. Ergothioneine was not an antagonist at either the DA- or ErA-sensitive receptor. The putative protein kinase C activators, 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) and 1,2-dioctanoyl-*sn*-glycerol (DiC_8), neither stimulated salivary fluid secretion nor potentiated the action of DA or ErN. The putative protein kinase C inhibitors, bisindolymaleimide (BIM) and calphostin C did not inhibit the action of DA or ErN, although low concentrations of calphostin C (10 nmol l^{-1}) appeared to potentiate the action of DA but not ErN. The ion transport inhibitors, furosemide and amiloride (both up to 1 mmol l^{-1}), had no significant effect on DA-stimulated or ErN-stimulated fluid secretion.

Key words: *Amblyomma hebraeum*, bromocriptine, dihydroergotamine, ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergonovine, ergothioneine, ergot alkaloids, methylergonovine, methysergide, tick salivary gland.

Introduction

Female ixodid ticks feed on a host for 7–14 days, during which time they concentrate the nutrient part of the blood meal by secreting a large volume of hyposmotic saliva. The total saliva secreted over the complete feeding period equals or exceeds the final engorged weight of the tick (reviewed by Kaufman, 1989; Sauer et al., 2000; Bowman and Sauer, 2004). Control of fluid secretion is mediated *via* at least three receptors. Dopamine (DA) and other catecholamines stimulate secretion *via* a DA receptor. This receptor appears to be in the D-1 family (Schmidt et al., 1981; Schmidt et al., 1982). γ-Aminobutyric acid (GABA) potentiates the fluid secretion stimulated by DA *via* a distinct receptor, but GABA has no intrinsic activity (Lindsay and Kaufman, 1986). The fact that muscimol, but not baclofen, mimics the potentiation of GABA, suggests that this receptor is of the $GABA_A$ type. The effect of GABA is blocked by picrotoxin (100 μmol l^{-1}) and (–)-bicuculline (100 μmol l^{-1}) in a non-competitive manner (Lindsay and Kaufman, 1986).

The ergot alkaloid (ErA), ergonovine (ErN), is also a very potent agonist of salivary fluid secretion (Kaufman and Wong, 1983). Results with selective antagonists demonstrate that ErN acts *via* a receptor distinct from the DA receptor (Kaufman and Wong, 1983). We call this third receptor an 'ErA-sensitive receptor' because we do not know the identity of its natural ligand.

Similar to other D-1 receptors, the effect of DA on this tissue is mediated by cAMP (Schmidt et al., 1981; Schmidt et al., 1982). Supramaximal concentrations of ErN also stimulate adenylate cyclase activity in the tick salivary glands, but

only to ~50% of the level stimulated by supramaximal concentrations of DA (W.R.K., unpublished observations), suggesting that the intracellular pathway mediating the action of ErN may be somewhat different from that of DA.

ErAs elicit an enormously broad spectrum of pharmacological effects, the best known being at adrenergic, dopaminergic and tryptaminergic receptors (Berde and Schild, 1978; Peroutka, 1996; Pertz and Eich, 1999), probably because the structures of these biogenic amines can be exactly superimposed onto specific parts of the ergoline ring structure (Berde, 1980). The purpose of this study was to extend our characterization of the pharmacological properties of the ErA-sensitive receptor in the tick salivary gland. We constructed dose–response curves for 11 ErAs available to us. Non-agonists and partial agonists were tested as putative inhibitors of the ErA and DA receptors. We also tested some inhibitors and activators of protein kinase C (PKC) for differential effects on the DA and ErN pathways. Likewise, we tested two inhibitors of ion transport (amiloride and furosemide) for differential effects on the two pathways.

Materials and methods

Ticks

All animal use in the Department of Biological Sciences, University of Alberta, is reviewed by the Biosciences Animal Policy and Welfare Committee according to the Guidelines established by the Canadian Council on Animal Care. *Amblyomma hebraeum* (Koch) were taken from a laboratory colony raised in darkness, at 26°C and high relative humidity. Ticks were fed on rabbits as originally described for *Dermacentor andersoni* (Kaufman and Phillips, 1973a). For this study we used partially fed ticks in the range of 80–300 mg (normal engorged mass of *A. hebraeum* is in the range of 1000–3000 mg).

Drugs and media

Modified Hank's saline (dissection medium)

Composition in g l^{-1} was: 11.5 NaCl, 1.6 D-glucose, 0.4 KCl, 0.14 $CaCl_2$, 0.098 $MgSO_4$, 0.06 KH_2PO_4, 0.05 $NaHPO_4$, 0.01 Phenol Red. The pH was adjusted with NaOH to 7.2; the osmotic pressure was ~360 mOsm.

Modified TC 199 (in-vitro bathing medium)

1 package powdered TC199 (11 g; Gibco, Grand Island, NY, USA), 2.1 g NaCl and 2.09 g 3-[*N*-morpholino]propanesulfonic acid (Mops; Sigma Chemical Co., St Louis, MO, USA) were dissolved in distilled-deionized water, the pH was adjusted with NaOH to 7.2 and the final volume was brought to 1 l.

The following drugs were a gift from Hoffman LaRoche (Basle, Switzerland): bromocryptine, methylergonovine, ergocristine, ergocristinine, ergocornine, ergocorninine and methysergide. (+/–)-Sulpiride (Ravizza, Italy or Delagrange, France) was a gift from Dr G. N. Woodruff, formerly of the University of Southampton, UK. The following drugs were purchased from Sigma: DA-HCl, ergonovine-maleate, dihydroergotamine, α-ergocryptine, ergothioneine and 5-hydroxytrypamine (5-HT). Structures of the ErAs used in this study [plus ergotamine from Kaufman (Kaufman, 1977)] are shown in Fig. 1. Deprenyl [monoamine oxidase (MAO) inhibitor] was purchased from Research Biochemicals Inc. (Natick, MA, USA). 1-oleoyl-2-acetyl-*sn*-glycerol (OAG), 1,2-dioctanoyl-*sn*-glycerol (DiC_8), bisindolymaleimide (BIM) and calphostin C, were purchased from Calbiochem (San Diego, CA, USA).

Stock solutions of all drugs were made daily; DA, deprenyl, ErN, methylergonovine, ergothioneine and methysergide were each dissolved in double-deionized water (to 1 or 10 mmol l^{-1}) and then diluted to working concentration in modified TC 199. Calphostin C, OAG, DiC_8, BIM, ergocryptine, bromocryptine, dihydroergotamine, ergocornine, ergocorninine, ergocristine, ergocristinine were each dissolved in dimethylsulphoxide (DMSO; 0.5 mmol l^{-1}, 1 mmol l^{-1}, 2 mmol l^{-1} or 10 mmol l^{-1}) and then diluted to working concentration in modified TC 199 such that the final concentration of DMSO did not exceed 1%; we have demonstrated previously that this concentration of DMSO does not significantly affect the rate of salivary fluid secretion *in vitro* (Lindsay and Kaufman, 1986).

In vitro technique for measuring salivary fluid secretion

The technique was similar to that described earlier (Kaufman and Phillips, 1973b; Wong and Kaufman, 1981). Glass Petri dishes (15 cm diameter) were lined with paraffin wax into which four fine glass posts were embedded to serve as anchors for the experimental glands. The Petri dishes were filled with liquid paraffin (light mineral oil; Fisher Scientific, Fair Lawn, NJ, USA). Briefly, salivary glands were dissected out under modified Hank's saline with their main ducts completely intact. The glands were transferred to modified TC 199 and extraneous tissue clinging to the main duct was teased away. Fine silk thread was ligated to a fragment of cuticle associated with the terminal portion of the main duct, but without occluding the orifice. The glands were then transferred to the Petri dish in a droplet of bathing medium such that the bathing medium adhered to the glass post and the silk thread draped over the far rim of the Petri dish. The thread was then gently pulled so that the main duct protruded from the bathing droplet into the mineral oil. Whenever the bathing medium contained an effective agonist, a small, spherical droplet of secreted fluid would appear and grow at the orifice of the main duct. For measuring the amount of secreted fluid, the droplet was removed from the orifice with a fine glass rod, allowed to sink, the diameter measured under a stereomicroscope fitted with an ocular micrometer, and the sphere volume calculated from the diameter of the droplet.

General experimental protocol

All experiments were done at room temperature (22.5±0.5°C). Glands were exposed to a control treatment (usually a given concentration of agonist), and the rate of secretion was recorded every 3–5 min until an equilibrated rate

for that treatment was observed (usually 10–20 min). During this time, the prevailing medium was changed for a fresh droplet every 3–5 min. Then the glands were exposed to successively higher concentrations of drug, each time an equilibrated rate of secretion being recorded following similar changes of prevailing medium. Concentration of drug was increased by 10-fold increments until the response was maximal. When the drug under consideration was not DA, the glands were then washed in drug-free TC 199 for at least 20 min, and then exposed to 10 μmol l^{-1} DA for three to five 3-min readings. Unless otherwise stated, the response of the glands to 10 μmol l^{-1} DA was designated as 100%, and the maximal responses of the other treatments were compared to this value.

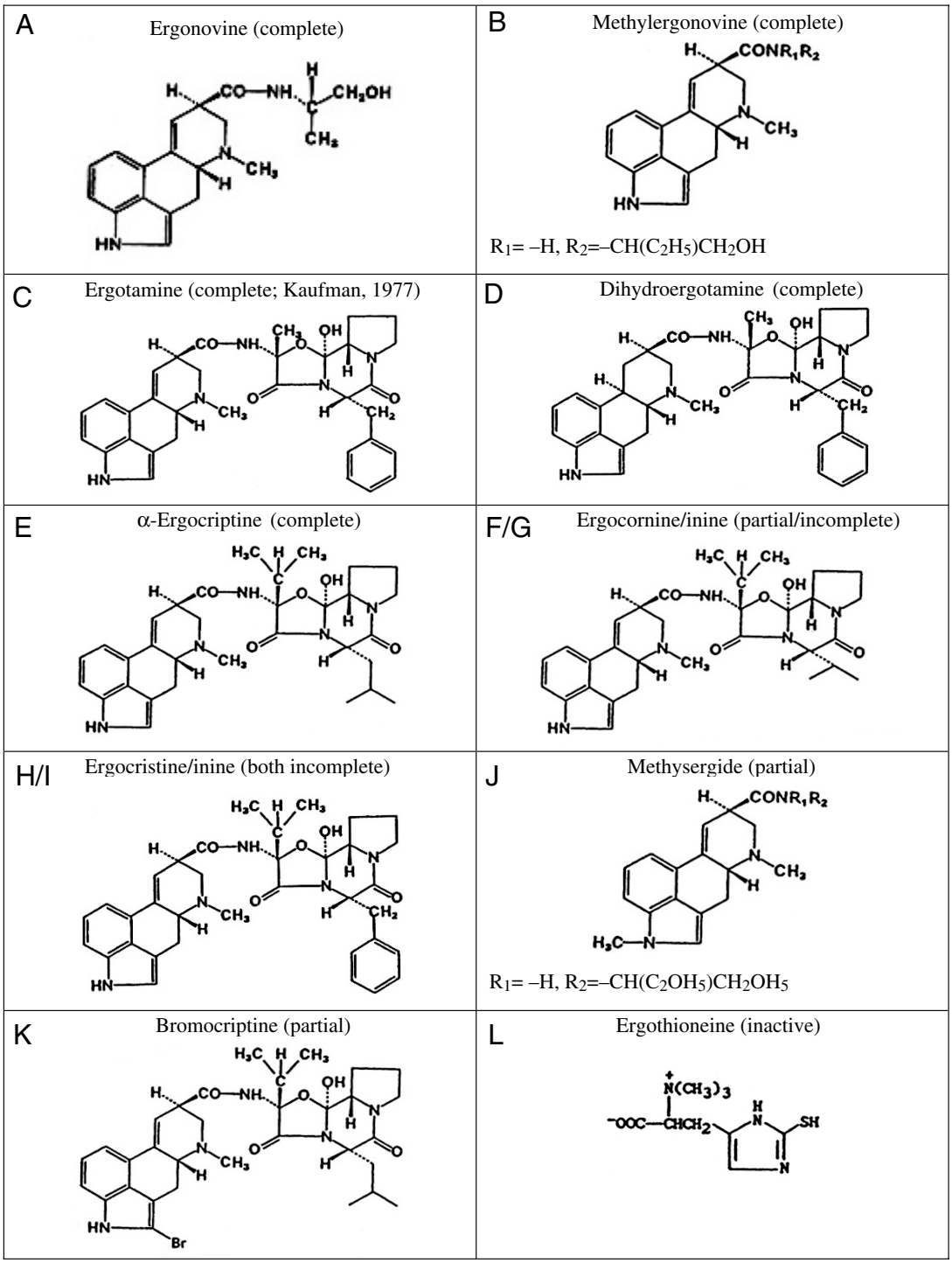

Fig. 1. (A–L) Ergot alkaloids tested on tick salivary glands. The agonist activity for each is indicated in parentheses. All structures compiled from Berde and Schild (Berde and Schild, 1978). Reproduced with kind permission of Springer Science and Business Media.

Putative antagonists were tested by one of two protocols. The first was a conventional dose–response protocol, in the presence of the drug being tested for antagonism. In the second protocol, the glands were exposed to an agonist drug (usually a sub-maximal concentration) until there was no further increase in secretory rate. Then putative antagonist was tested (several concentrations) until an equilibrated rate of secretion was observed at each concentration. To test for reversal of antagonism, the glands were re-exposed to agonist alone for up to 30 min.

Inhibitory constants (K_i) were calculated from the following equation:

$$K_i = (\text{dose ratio} - 1) / [I] ,$$

where dose ratio is the ratio of the two agonist concentrations producing the same response in the presence and absence of the inhibitor, I (Goldstein et al., 1974).

Statistics

Unless otherwise stated, data are reported as mean ± s.e.m. (*N*), and statistically analysed using one-way ANOVA or Student's *t*-tests.

Results

Agonists

The 11 ErAs tested in this study (Fig. 1) can be categorized arbitrarily into four groups on the basis of the responses obtained. (1) *Complete agonists* (Fig. 2) were those that stimulated the maximum response at or below the highest concentration tested. These were, in order of potency (approximate concentration eliciting 50% maximum response in parentheses): dihydroergotamine (0.02 μmol l^{-1}), ErN (0.06 μmol l^{-1}), methylergonovine (0.1 μmol l^{-1}), α-ergocryptine (0.9 μmol l^{-1}). (2) *Incomplete agonists* were those that neither elicited the maximum response nor appeared to reach some plateaued response at the highest concentration that could be tested (usually because of limited availability). The approximate concentrations eliciting 20% maximum response were: ergocorninine (3.5 μmol l^{-1}), ergocristinine (7.5 μmol l^{-1}), and ergocristine (10 μmol l^{-1}; Fig. 3). (3) *Partial agonists* (Fig. 4) were those for which the response plateaued at substantially less than 100% of the maximum response. These were (with the approximate lowest concentration eliciting the respective maximum response in parentheses): ergocornine (50% maximum by 1 μmol l^{-1}), methysergide (28% maximum by 10 μmol l^{-1}) and bromocryptine (22% maximum by 10 μmol l^{-1}). (4) *Non-agonists.* Ergothioneine was the only ErA with no intrinsic activity at the highest concentration tested (seven glands tested up to 1 mmol l^{-1}).

As mentioned in the Introduction, ErAs interact frequently with 5-HT and catacholamine receptors, behaving either as agonists or antagonists, but the natural ligand for the ErA-sensitive receptor in tick salivary glands has not yet been identified. The effect of 5-HT on tick salivary glands has been tested in a number of studies, and at best it is a very weak agonist (Kaufman and Phillips, 1973b; Kaufman, 1977; Needham and Sauer, 1975). We tested its action again as part of this study, with the hope of determining whether it acted *via* the DA receptor (blocked by butaclamol) or the ErA-sensitive receptor [blocked by sulpiride (Kaufman and Wong, 1983)]. Of 16 glands tested that responded to 10 μmol l^{-1} DA, only five were also stimulated by 5-HT (up to 10 mmol l^{-1}); 5-HT was an incomplete agonist, with 20% maximum response occurring at ~220 μmol l^{-1} (Fig. 3).

We asked whether this inconsistent and weak response to 5-HT might be due to high levels of monoamine oxidase (MAO) in the tissue. So we repeated some dose–response experiments with 5-HT in the presence of 220 μmol l^{-1} deprenyl, an MAO inhibitor known to be effective on tick salivary glands (Kaufman and Sloley, 1996). Even though MAO should have

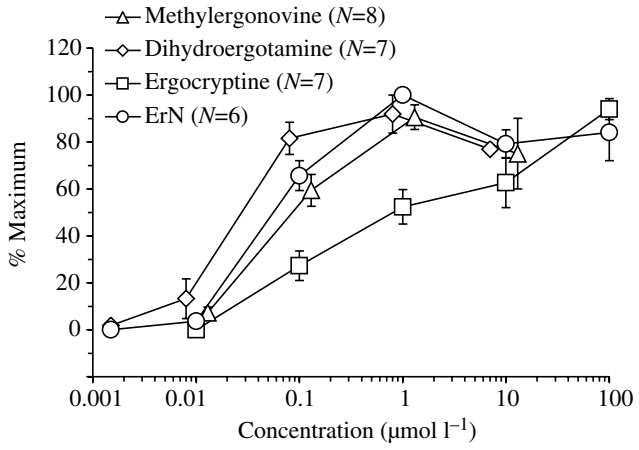

Fig. 2. Complete agonists. Dose–response curves for the indicated drugs were constructed as described in Materials and methods. The standard error of the mean (s.e.m.) is shown wherever it exceeds the size of the symbol.

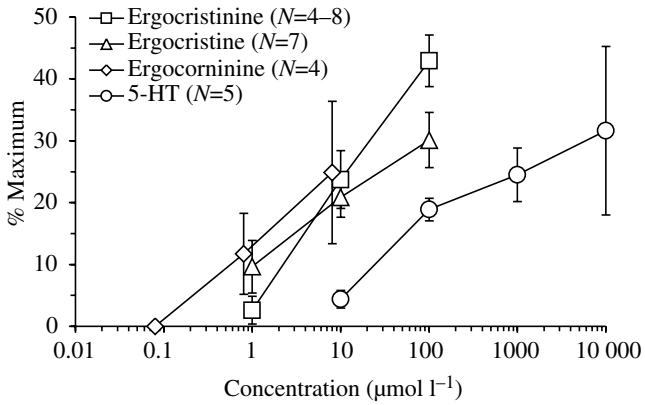

Fig. 3. Incomplete agonists. Dose–response curves for the indicated drugs were conducted as described in Materials and methods. The s.e.m. is shown wherever it exceeds the size of the symbol. The data for 5-hydroxytrypamine (5-HT) includes only those glands that showed some response to 5-HT.

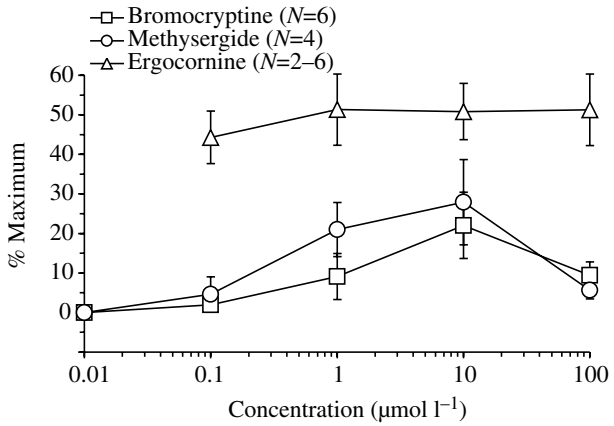

Fig. 4. Partial agonists. Dose–response curves for the indicated drugs were conducted as described in Materials and methods. The s.e.m. is shown wherever it exceeds the size of the symbol.

been almost completely inhibited under these conditions, 5-HT still failed to stimulate salivary fluid secretion in eight glands, all of which responded to 10 μmol l^{-1} DA (data not shown). Thus we abandoned our attempt to determine at which receptor 5-HT elicits its weak, inconsistent effect.

Putative antagonists

Ergothioneine (non-agonist), bromocryptine and methysergide (partial agonists) were tested for their ability to inhibit ErN- and DA-stimulated secretion. Ergothioneine (1 mmol l^{-1}) caused no rightward shift of the ErN dose–response curve (Fig. 5) nor of the DA dose–response curve (five trials; data not shown). Bromocryptine (3 trials) and methysergide (3 trials) did not significantly antagonize the action of DA (Fig. 6A), but both were effective, surmountable antagonists of ErN (Fig. 6B), with K_is of ~0.3 μmol l^{-1} (bromocryptine) and ~11 μmol l^{-1} (methysergide).

DA and ErN have been demonstrated to act *via* distinct receptors by the differential antagonism of sulpiride (Kaufman and Wong, 1983). We confirmed these findings here, determining an inhibitory constant of ~0.73 μmol l^{-1} for sulpiride on ErN (Fig. 5), and an inability for sulpiride to attenuate the action of DA (data not shown).

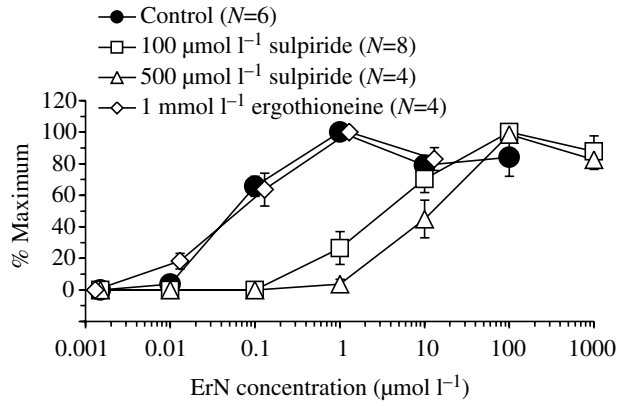

Fig. 5. Inhibition of ergonovine (ErN) by putative antagonists, ergothioneine and (+/–)-sulpiride. The s.e.m. is shown wherever it exceeds the size of the symbol.

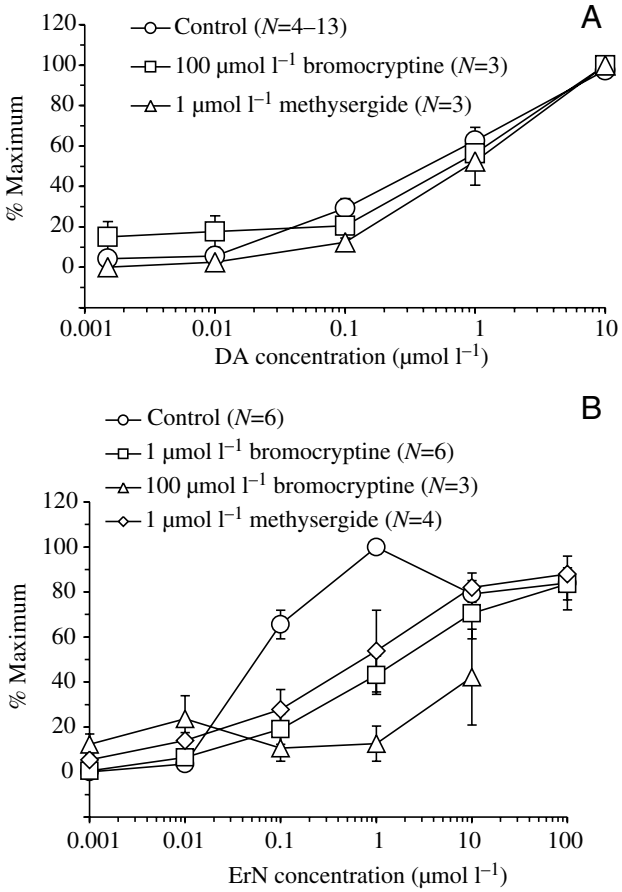

Fig. 6. Inhibition of (A) dopamine (DA) and (B) ergonovine (ErN) by the partial agonists, bromocriptine and methysergide. The s.e.m. is shown wherever it exceeds the size of the symbol.

Intracellular pathways

The intracellular pathways mediating DA-induced secretion involve cAMP, Ca^{2+}, cytosolic phospholipase A_2 (cPLA_2), and arachidonic acid (reviewed by Sauer et al., 2000; Bowman and Sauer, 2004). Nothing is known about the intracellular pathways mediating ErA-induced secretion. However, there is evidence that protein kinase C (PKC) is a component of the intracellular signalling pathway in tick salivary glands (see Discussion). So, in this study we tested the actions of a number of drugs known to stimulate or inhibit PKC in other systems to learn whether the action of DA and ErN were affected differently.

BIM (Toullec et al., 1991) and calphostin C (Kobayashi et al., 1989; Bruns et al., 1991) were tested for possible inhibitory effects, and OAG (Benz et al., 1992; Florin-Christensen et al., 1993) and DiC_8 (Lapetina et al., 1985) were tested for possible excitatory effects on DA- and ErA-

stimulated fluid secretion. Salivary glands were exposed to a sub-maximal concentration of DA (0.1 μmol l^{-1}) or ErN (0.1 μmol l^{-1}) for six 5-min readings to determine the equilibrated response at that concentration; the mean value (± s.e.m.) of the last two or three readings was recorded as the control response. Then the test drug (PKC activator or inhibitor) was added in the presence of DA or ErN and secretion was monitored for up to a further six 5-min readings. The glands were then re-exposed to either DA or ErN alone to record recovery. The results for the putative PKC activators, OAG and DiC_8, are presented in Table 1, and those for the putative PKC inhibitors, BIM and calphostin C, are presented in Table 2 and Fig. 7.

Neither OAG nor DiC_8 potentiated the action of sub-maximal concentrations of DA or ErN (Table 1). In all cases the secretory rates declined in the presence of OAG or DiC_8 and on return to the control treatment, but at least part of this reduction may have been the natural decline that occurs with time when glands are continually stimulated for extensive periods (Kaufman, 1976).

BIM did not inhibit the secretory response of salivary glands in the presence of sub-maximal (0.1 μmol l^{-1}) or supra-maximal (10 μmol l^{-1}) concentrations of ErN or DA (Table 2) The response to calphostin C depended on the concentration range. Low concentrations of calphostin C (10 nmol l^{-1}) appeared to potentiate the action of DA (leftward shift of the dose-response curve; Fig. 7A) but not that of ErN (Fig. 7B). At 0.1 μmol l^{-1} calphostin C, there was no statistically significant inhibitory (or potentiating) effect using a sub-maximal (0.1 μmol l^{-1}) concentration of ErN or DA (Table 2).

Table 1. *Effect of the protein kinase C activators on dopamine- and ergonovine-stimulated fluid transport by tick salivary glands*

Treatment	% Maximum*	*N*	*P*†
OAG			
DA (control)	85.1±3.8	8	–
DA + OAG	79.9±4.4	8	0.382
Return to DA	63.8±4.4	8	0.002
ErN (control)	87.5±2.2	7	–
ErN + OAG	72.4±9.9	7	0.183
Return to ErN	55.4±10.8	7	0.025
DiC_8			
DA (control)	78.6±3.5	8	–
DA + DiC_8	50.6±6.0	10	0.001
Return to DA	43.4±6.5	4	0.005
ErN (control)	78.6±4.8	9	–
ErN + DiC_8	59.2±3.0	9	0.004
Return to ErN	41.6±2.9	9	0.0000

OAG, 1-oleolyl-2-acetyl-*sn*-glycerol (100 μmol l^{-1}); DiC_8,1,2-dioctanoyl-*sn*-glycerol (100 μmol l^{-1}); DA, dopamine (0.1 μmol l^{-1}); ErN, ergonovine (0.1 μmol l^{-1}).

*% Maximum is calculated from the maximum rate recorded during each initial control period (± s.e.m.).

†Student's *t*-test (cf control).

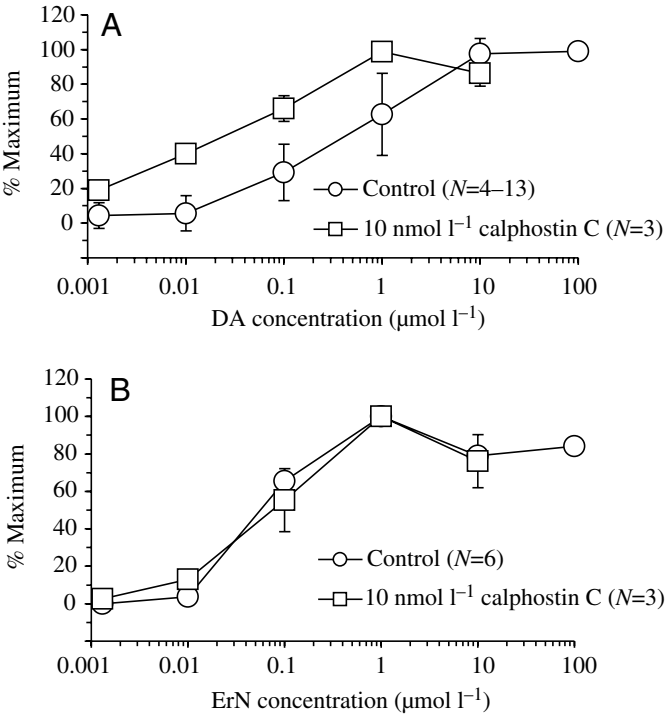

Fig. 7. Potentiation of (A) dopamine (DA) but not (B) ergonovine (ErN) by the putative PKC inhibitor calphostin C. The s.e.m. is shown wherever it exceeds the size of the symbol.

Ion transport inhibitors

Furosemide [an inhibitor of various Cl^- cotransporter systems (Cabantchik and Greger, 1992)] and amiloride [an inhibitor of many Na^+ transport systems (Kleyman and Cragoe, Jr, 1988)] were both tested for effects on DA- and ErN-stimulated secretion. Salivary glands were exposed to 10 μmol l^{-1} DA or 10 μmol l^{-1} ErN until maximum secretion was achieved. They were then exposed to DA or ErN along with 1 μmol l^{-1} to 1 mmol l^{-1} furosemide or amiloride for three successive readings of 5 min for each concentration. Neither furosemide nor amiloride had any significant effect on DA- or ErN-stimulated secretory rates; note, however, that the sample sizes were small (Table 3).

Discussion

The multiple agonist and antagonist actions of ErAs in systems other than the tick salivary gland (most studies are on mammals) are mediated predominantly, if not exclusively, *via* various catecholamine and tryptamine receptors (Berde and Schild, 1978; Goldstein et al., 1980; Pertz and Eich, 1999). Most studies on insects and other invertebrates conform to vertebrate models in this respect (e.g. Sakharov et al., 1989; Wedemeyer et al., 1992; Blenau et al., 1998; Degen et al., 2000). However, 5-HT is only a very weak agonist at best for stimulating fluid secretion in tick salivary glands (Kaufman and Phillips, 1973b; Needham and Sauer, 1975; Kaufman, 1977; this study). Evidence for a DA receptor (a D-1 type),

Table 2. *Effect of the protein kinase C inhibitors, bisindolymaleimide and calphostin C on ergonovine- and dopamine-stimulated fluid transport by tick salivary glands*

Treatment	% Maximum*	*N*	*P*†
BIM			
10 μmol l^{-1} ErN (control)	82.3±3.1	5	–
10 μmol l^{-1} ErN + 0.1 μmol l^{-1} BIM	79.9±3.0	5	0.589
10 μmol l^{-1} ErN + 0.3 μmol l^{-1} BIM	93.5±10.4	5	0.343
10 μmol l^{-1} ErN + 1.0 μmol l^{-1} BIM	79.3±11.2	4	0.810
0.1 μmol l^{-1} ErN (control)	82.7± 2.0	4	–
0.1 μmol l^{-1} ErN + 0.2 μmol l^{-1} BIM	105±14.6	4	0.227
Return to 0.1 μmol l^{-1} ErN	66.0±3.2	3	0.015
0.1 μmol l^{-1} DA (control)	87.2±4.8	4	–
0.1 μmol l^{-1} DA + 0.2 μmol l^{-1} BIM	97.1±14.4	4	0.554
Return to 0.1 μmol l^{-1} DA	108±7.8	3	0.095
Calphostin C			
0.1 μmol l^{-1} ErN (control)	83.9±2.9	5	–
0.1 μmol l^{-1} ErN + 0.1 μmol l^{-1} calphostin C	90.5±3.6	5	0.194
Return to 0.1 μmol l^{-1} ErN	48.9±0.5	2	0.0002
0.1 μmol l^{-1} DA (control)	82.7±4.1	6	–
0.1 μmol l^{-1} DA + 0.1 μmol l^{-1} calphostin C	106±10.1	6	0.077
Return to 0.1 μmol l^{-1} DA	104±17.7	5	0.398

BIM, bisindolymaleimide; DA, dopamine; ErN, ergonovine.

*% Maximum is calculated from the maximum rate recorded during each initial control period (± s.e.m.).

†Student's *t*-test (cf control).

however, is considerable (for reviews, see Sauer et al., 2000; Bowman and Sauer, 2004). As mentioned in the Introduction, ErAs do not exert their effects *via* this receptor. In brief, the ErA effects on the tick salivary gland cannot be interpreted in the same light as ErA effects in most other systems. There seem to be only very few other examples in the literature in which an ErA might act *via* an unidentified receptor (den Boer et al., 1991).

In this study, the ErAs tested fell into one of four classes, grouped arbitrarily according to their efficacy at the ErA-sensitive receptor: complete agonists, incomplete agonists, partial agonists and non-agonists. The structures of the ErAs tested are shown in Fig 1. Those that exhibited at least some agonist activity (all except ergothioneine) are based on an ergoline ring structure. Although ergotamine was not tested here, it has also been reported as a full agonist, with a potency similar to that of ergonovine (Kaufman, 1977). There is little beyond that broad generalization, however, to explain the other differences in activity presented here. For example, only three of the five complete agonists are ergopeptines (ergotamine, dihydroergotamine and α-ergocriptine), the other two (ErN and methylergonovine) are not. Moreover, with the exception of methysergide, all of the incomplete and partial agonists are also ergopeptines (ergocornine, ergocorninine, ergocristine, ergocristinine and bromocriptine).

It is commonly observed that the action of ErAs is difficult to reverse even with extensive washout procedures (Bond et al., 1989; Schöning et al., 2001), and this was found to be the case with the tick salivary gland. One potential explanation for the long-lasting effect is continual diffusion from a pool of drug absorbed into the tissue during the incubation period (Bulow et al., 1986; Tfelt-Hansen and Johnson, 1993). Berridge and Prince (Berridge and Prince, 1973), reporting on the agonist action of LSD at the 5-HT receptor of the blowfly salivary gland, discounted the latter possibility for that system. They proposed instead that part of the ErA molecule (presumably the diethyl amide) binds tightly to an allosteric site of the 5-HT receptor, allowing the active portion (presumably the ergoline) to repeatedly attach to and disengage from the active site. Similar to the blowfly salivary gland, the tick salivary gland is free of layers of muscle or connective tissue that could serve to store a pool of drug (although extrinsic fat body tissue is omnipresent). In rat tail artery preparations, insurmountable blockade of the 5-HT response by methysergide has been attributed to allosteric modulation of 5-HT_{2A} receptors rather than to pseudoirreversible inhibition (Pertz and Eich, 1992). In this case, however (unlike that for the blowfly and tick salivary glands), onset of action is also very prolonged (Schöning et al., 2001). A general model has been proposed whereby one can differentiate between slow ligand–receptor dissociation from slow diffusion (Martin et al., 1995).

The results presented here further support earlier observations that 5-HT is unlikely to be the endogenous ligand at the ErA-sensitive receptor. The small and inconsistent effect of 5-HT might be due to an indirect action. For example, tick salivary glands contain a relatively high endogenous content of DA (Kaufman and Sloley, 1996), possibly in the numerous granular cells. Perhaps the inconsistent agonist action of 5-HT

Table 3. *Effect of amiloride and furosemide on dopamine- and ergonovine-stimulated fluid transport by tick salivary glands*

Treatment	% Maximum*	*N*	*P*†
Amiloride			
DA (control)	100±0	6	–
DA + 1 μmol l^{-1} amiloride	108±6.9	3	0.369
DA + 10 μmol l^{-1} amiloride	104±15.0	3	0.832
DA + 100 μmol l^{-1} amiloride	99.8±11.1	6	0.986
DA + 1 mmol l^{-1} amiloride	98.4±13.6	2	0.925
DA + 10 mmol l^{-1} amiloride	83.7±28.3	2	0.667
ErN (control)	100±0	2	–
ErN + 1 μmol l^{-1} amiloride	112±22.6	2	0.701
ErN + 10 μmol l^{-1} amiloride	112±12.4	2	0.500
ErN + 100 μmol l^{-1} amiloride	94.3±15.3	2	0.642
Furosemide			
DA (control)	100±0	2	–
DA + 1 μmol l^{-1} furosemide	123±11.8	2	0.218
DA + 10 μmol l^{-1} furosemide	134±0	2	–
DA + 100 μmol l^{-1} furosemide	122±0	2	–
DA + 1 mmol l^{-1} furosemide	131±3.1	2	0.063
ErN (control)	100±0	3	–
ErN + 1 μmol l^{-1} furosemide	104±2.7	3	0.190
ErN + 10 μmol l^{-1} furosemide	102±2.3	3	0.765
ErN + 100 μmol l^{-1} furosemide	96.8±9.4	3	0.684

DA, dopamine (10 μmol l^{-1}); ErN, ergonovine (10 μmol l^{-1}).

*% Maximum is calculated from the maximum rate recorded during each initial control period (± s.e.m.).

†Student's *t*-test (cf control).

is due to an occasional stimulation of DA release from this endogenous source.

There is now a growing understanding about the intracellular signalling cascade following stimulation of the tick (*Amblyomma americanum*) salivary gland DA receptor (reviewed by Sauer et al., 2000; Bowman and Sauer, 2004). The salivary gland contains several isoforms of a cAMP-dependent protein kinase ($cAPKC_{1\text{-}3}$) that are activated in isolated salivary glands exposed to DA. As a result, at least a dozen salivary gland proteins are phosphorylated. The glands also contain phosphoprotein phosphatase activity, further supporting a role for PKC in salivary fluid secretion. Under the conditions tested here, there is little evidence to suggest that PKC is an intermediate in the pathway linked to the ErA-sensitive receptor or the DA receptor. The two PKC activators, OAG and DiC_8, did not stimulate salivary fluid secretion on their own (data not shown), and they did not potentiate the stimulatory effect of sub-maximal concentrations of DA or ErN (both at 0.1 μmol l^{-1}; Table 1). The PKC inhibitors, BIM and calphostin C, likewise did not inhibit the stimulatory action of 0.1 μmol l^{-1} DA or ErN (Table 2); if anything, the trend was to potentiate the action of DA and ErN, though the differences between control and treated samples in Table 2 were not statistically significant in a consistent manner. The potentiating trend was also observed at lower concentrations of calphostin C, where 10 nmol l^{-1} appeared to shift the dose–response curve of DA to the left, but not that of ErN (Fig. 7); the number of replicates in this experiment, however, was small. This matter clearly merits further investigation.

We have some confidence that the experimental protocol used for the PKC experiments was appropriate, both with regard to the concentration ranges tested and the duration of incubation. Just a few examples include: PKC mediation in 5-HT stimulated ciliary beat frequency in embryos of the pond snail, *Heliosoma trivolvis* (Christopher et al., 1999), inhibition of PKC by BIM in chick proximal tubule (Dudas et al., 2002), activation of PKC in rabbit portal vein myocytes (Albert and Large, 2001), stimulation of PKC of human kidney proximal tubules (Pietig et al., 2001), and inhibition of PKC by calphostin C on human endothelial cells (Orzechowski et al., 2001).

Our knowledge of the pharmacological properties of the tick ErA-sensitive receptor is obviously rudimentary compared to that of many mammalian systems, at least in part because relatively few congeners have been tested. Recently it has been shown that 8R-lisuride is a partial agonist at human histamine H_1 receptors (Bakker et al., 2004), which expands the repertoire of ErA targets yet further. However, histamine (up to 1 mmol l^{-1}) did not stimulate fluid secretion in tick salivary glands (Kaufman, 1977). Unfortunately, we still have no idea as to what the natural ligand is at the tick salivary gland ErA-sensitive receptor, or whether it is a component of a neuronal or hormonal pathway. We also do not know the physiological conditions under which this pathway to fluid secretion is stimulated [the DA pathway seems to be involved in

haemolymph volume regulation (Kaufman et al., 1980)]. This remains a major gap in our understanding of tick salivary gland function.

List of abbreviations

5-HT	5-hydroxytrypamine
BIM	bisindolymaleimide
$cPLA_2$	cytosolic phospholipase A_2
DA	dopamine
DiC_8	1,2-dioctanoyl-*sn*-glycerol
ErA	ergot alkaloid
ErN	ergonovine
GABA	γ-aminobutyric acid
MAO	monoamine oxidase
OAG	1-oleoyl-2-acetyl-*sn*-glycerol
PKC	protein kinase C

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