ly. However, the least pesticide performance was associated with the insecticide compound Chloropyrifos when used at the same level of the tested IGRs (5 kg/acre). In general the soil mites are more susceptible to the tested IGRs than chlorpyrifos at 5 kg/acre which was reported as a high potent soil pesticide[14].

Closer examination of the detailed data (table 3) of treated plots revealed that the reduction percentages in natural population of mite was slightly different in both levels of vertical distribution, which could be explained in view of the behaviour of the pesticide used. However, RO-10-3108 and Chlorpyrifos at the rate of 5 kg/acre performed better in the surface stratum than in the subsurface-stratum which means more adsorption in this layer and less leaching. Increasing the rate of Chlorpyrifos 4 times did permit slight leaching to the subsurface stratum only and a subsequent slight increase in efficacy (70.5%) reduction as compared with 67.7% reduction in the surface stratum).

Such findings could lead us to make some restrictions in chosing the soil pesticides to fit with our requirements. The main tool in this respect must be the behaviour of soil pesticides under different environmental conditions, including soil type, in relation to behaviouristic abundance of the target organisms.

It is impossible to make generalizations about the efficacy of the different tested pesticides with regard to the mite sub-orders under investigation. However, sub-orders specificity relationships could easily be detected. For example, although Astigmata was the most susceptible sub-order to all tested compounds, its rank of susceptibility could be arranged in descending order as follow: RO-08-9801, Diflubenzuron, Chlorpyrifos (20 kg), RO-10-3108 and Chlorpyrifos (5 kg) respectively. Other sub-order, such as the Heterostigmata, exhibited a different rank of response, revealing Chlorpyrifos (20 kg), Diflubenzuron, RO-08-9801, RO-10-3108, and Chlorpyrifos (5 kg) to be the most effective. However, it is of interest to note here that the 3 IGRs RO-08-3108, RO-10-3108 and Diflubenzuron at 5 kg/acre induced significant reduction, comparable with that obtained by the organophosphorous compound Chlorpyrifos at 20 kg/acre especially against the sub-orders Astigmata, Mesostigmata and Prostigmata. Furthermore, in particular the Heterostigmata were highly susceptible to the juvenoids RO-08-3108 and RO-10-3108 and to the disruptor of the chitin biosynthesis, Diflubenzuron if short-term (0–7 days posttreatment) efficiacy was considered (table 4).

Our results indicate clearly the important role which IGRs could play in depressing the population of soil mites. However, much higher dosages must be tested in order to obtain a high degree of control. Also special care must be taken to consider the specific response of the predaceous species of soil mites (belonging to different groups) to such class of chemicals.

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Specific inhibition of a calcium dependent activation of brain cyclic AMP phosphodiesterase activity by vinblastine

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Summary. Vinblastine selectively inhibits the activation of brain cyclic AMP phosphodiesterase activity by Ca^{++}-protein activator (50% inhibition by 2×10^{-5} M). This inhibitory effect was reversed by excessive amounts of the activator, whereas large quantities of Ca^{++} caused only a slight suppression of the vinblastine effect. This result of vinblastine suggests a new site of its action and also suggests the possible role of protein activator, phosphodiesterase proteins or cyclic nucleotides in the previously known effects of vinblastine in vivo and in vitro.

Vinblastine (VB) inhibits the assembly of microtubules and is also known to interact with systems affected by Ca^{++}[2,3]. High concentrations of Ca^{++} or VB precipitate tubulin, a subunit of microtubules, as well as other acidic proteins[2,4]. In the presence of Ca^{++}, dose-effect curve for precipitation of protein by VB shifted toward lower values[2]. VB was reported to inhibit Ca^{++}-induced release of catecholamine from adrenal gland[3]. Therefore, it is possible that Ca^{++} and VB may act at the same binding site on certain proteins[2,3].

Recently, we observed that brain supernatant protein(s) precipitated by a high concentration of VB has cyclic AMP phosphodiesterase (PDE) activity and this specific activity is about 3 times as high as that found in the original supernatant[5]. The predominant PDE in the brain supernatant is the activity which can be stimulated by an endogenous calcium dependent protein activator (PA), a heat stable acidic protein[6,7]. Following binding of Ca^{++} to PA, this complex binds to PDE with subsequent activation[8,9]. We have investigated in this paper the effect of VB on the Ca^{++}-PA regulated PDE activity of brain supernatant with special reference to the possible site of its action.

Materials and methods. VB was a generous gift from Eli Lilly Co., Indianapolis, Indiana (Dr R.J. Hosley). The crude brain supernatant PDE activity was prepared from rat brain. The rat brain was homogenized with 2 vol. of 10 mM imidazole buffer, pH 6.9, containing 1.5 mM $MgCl_2$, and following centrifugation at $70{,}000 \times g$ for 30 min, the supernatant was used as a source of enzyme activity. PA-free PDE was prepared from bovine brain by a slight modification of the method of Cheung and Lin[10]. Purification of PA was done as reported by Teo et al.[11].

PDE activity was measured by the method of Thompson and Appleman[12] as modified by Boudreau and Drummond[13]. The incubation mixture contained 1 µM of ^{3}H-cyclic AMP, 2 mM $MgCl_2$ 0.02% albumin and 50 mM tris-HCl buffer, pH 7.4, in a final volume of 250 µl. The reaction was stopped by immersing the tube in boiling water for 2 min. Ca^{++} and ethyleneglycol-bis (β-amino-ethyl ether) N,N'-tetraacetic acid (EGTA) were added as indicated.

Results and discussion. The dose-response effect of VB on the Ca^{++}-activated crude brain supernatant PDE activity showed 50% inhibition by $6\text{–}8\times10^{-5}$ M (figure 1). However, in the presence of 0.1 mM EGTA alone, inhibition did not exceed 50% even by 5×10^{-4} M VB. When PA-free PDE was activated by the additions of 0.1 µg of PA and 0.1 mM Ca^{++}, 2×10^{-5} M VB caused 50% inhibition. If the VB effect was measured in the presence of EGTA, $1.25\text{–}2.5\times10^{-5}$ M VB slightly stimulated PA-free PDE activity (8–19%) (not shown). The relationship of VB and Ca^{++} with PA was further investigated. If VB competes with Ca^{++} at the Ca^{++} binding sites of PA, large quantities of Ca^{++} may reverse the inhibitory effect of VB. Or, if VB blocks the action of PA or PA-Ca^{++} complex, large amounts of PA or PA-Ca^{++} complex may reverse the VB effect. The results of such experiments using PA-free PDE are shown in figures 2 and 3. In the presence of 0.1 mM Ca^{++}, increasing the PA concentration reduced the inhibitory effect of VB and the effect of VB was completely abolished by 6.3 µg of PA (figure 2). When the boiled extract was used as the source of PA, 160 µg of the extract was necessary to abolish the VB effect[14]. Increasing concentrations of Ca^{++} up to 4 mM in the presence of 0.1 µg of PA caused only a slight suppression of the inhibitory effect of VB (figure 3). Although VB inhibited the Ca^{++}-PA regulated brain supernatant PDE activity, VB does not appear to compete with Ca^{++} for its binding sites on PA (figures 1 and 3). Since the VB inhibitory effect was abolished by increasing amounts of PA, in the presence of Ca^{++}, the effect of VB on Ca^{++}-PA activated PDE activity appears to be due to interference with the function of PA. Detailed mechanism(s) by which VB may interfere with the function of PA is not known. Possible mechanisms are: binding of VB to PA at a site different from that for Ca^{++} binding, or to PDE at a site apart from catalytic site (PA-Ca^{++} complex binding site on PDE). There seems to be another possibility that VB may act on the Ca^{++}-PA-PDE complex.

The reason for the slight reduction of VB effect by large amounts of Ca^{++} is not known at present time.

The concentration of VB causing 50% inhibition of Ca^{++}-PA activated PDE activity of bovine brain is similar to the one that causes 50% binding of bovine brain tubulin[15]. A similar concentration of VB causes 50% binding of VB to low affinity VB binding site of rat brain tubulin and seems to be responsible for the aggregation of microtubules[16]. Furthermore, agents other than VB, such as antipsychotic phenothiazines and Zn^{++}, also cause both selective inhibition of Ca^{++}-PA regulated brain PDE activity[17,18] and

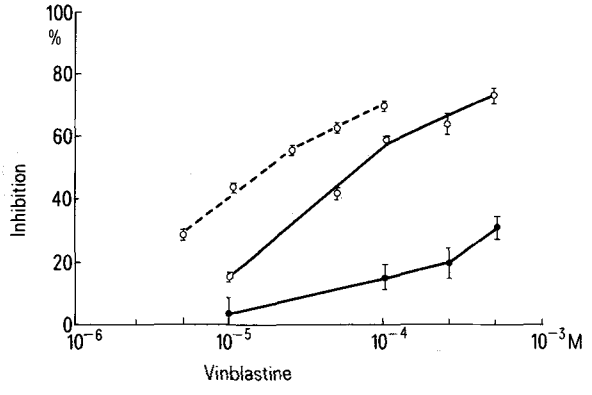

Fig. 1. The dose-effect curve of vinblastine on the brain supernatant cyclic AMP-PDE activity. Values are means ± SEM of 6–12 determinations. ●——●, Crude brain supernatant PDE activity measured in the presence of EGTA; ○——○, crude brain supernatant PDE activity activated by Ca^{++} (0.125 mM) in the presence of EGTA (0.1 mM); ○– – –○, partially purified PA-free PDE activity activated by Ca^{++} (0.1 mM) plus PA (0.1 µg/assay). No EGTA in this experiment.

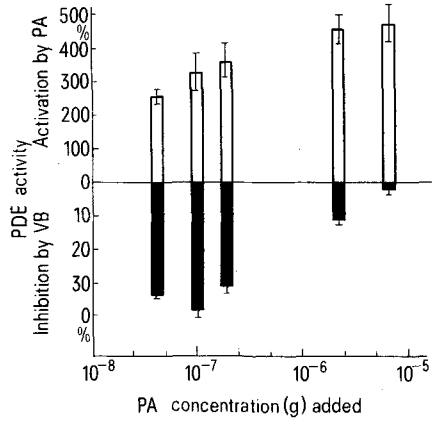

Fig. 2. The effects of various amounts of PA on the PDE activity and on the inhibitory potency of vinblastine. Values are means ± SEM of 6 determinations. The effects of varying concentrations of either PA alone or PA in the presence of vinblastine (1.25×10^{-5}) were examined at fixed Ca^{++} concentration (0.1 mM). Experiment was performed using partially purified brain PA-free PDE and purified PA. EGTA was not added in this experiment. Y-axis is an activation of PDE activity in the presence of increasing amounts of PA (upward) or an inhibition of PDE activity by vinblastine in the presence of increasing amounts of PA (downward).

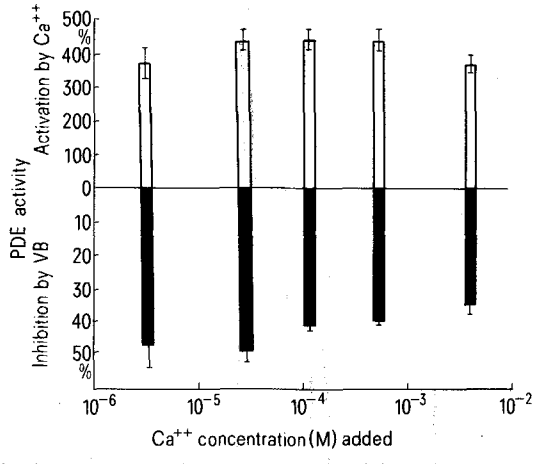

Fig. 3. The effects of various amounts of Ca^{++} on the PDE activity and on the inhibitory potency of vinblastine. Values are means ± SEM of 6 determinations. The effects of varying concentrations of either Ca^{++} alone or Ca^{++} in the presence of vinblastine (1.25×10^{-5} M) were examined at fixed PA concentration (0.1 µg/assay). Y-axis is an activation of PDE activity in the presence of increasing amounts of Ca^{++} (upward) or an inhibition of PDE activity by vinblastine in the presence of increasing amounts of Ca^{++} (downward). Other experimental conditions were same as figure 2.

inhibition of microtubule polymerization or production of special aggregation of microtubules[19,20]. The associations of PA-Ca^{++} activable PDE activity and protein kinase activities with microtubules[5], and of PA with mitotic apparatus[21] are also reported. Together, these data seem to suggest the interactions between microtubule, and PDE, PA-Ca^{++} complex or possibly cyclic nucleotides. Although the characteristics and functions of these interactions are not well understood, recent reports showed the participations of PA and cyclic AMP in the regulation of microtubule polymerization[22-24].

Since the concentration of PA was found to modulate the inhibitory effect of VB, varying effectiveness of VB as an antineoplastic agent[25] may partly depend upon the level of PA, PA-Ca^{++} activable PDE or cyclic nucleotides in tumor cells. Changes in these parameters are known to occur in tumor cells[26-28].

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Effect of para-methoxyphenylethylamine on chronic stress-induced hypertension in the rat[1]

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Summary. This report presents data showing that para-methoxyphenylethylamine is effective in both preventing and reversing chronic stress-induced hypertension in the rat.

In 1932, Epstein et al.[2] showed that the phenylethylamine derivative para-methoxyphenylethylamine (PMPEA) produced a marked pressor response when acutely injected into the decerebrate cat preparation. These authors also showed PMPEA to be acutely pressor in the vagotomized rabbit preparation. It was not until 1970 that Walker et al.[3] reported that the action of PMPEA on blood pressure was independent of its stimulant action on spinal monosynaptic reflex transmission. During that long interim period, much interest was paid to the derivatives of phenylethylamine with their implication in the basic mechanism(s) of action of several groups of centrally active compounds[4-6], being specifically referred to as a class of compounds known collectively as microamines[7], and finally, the evolvement of a theory of their role in the pathogenesis of affective behavior[8], or more specifically, modulation of synaptic transmission[9].

Recent work in our laboratories has shown PMPEA to have sexual stimulant properties in both the male[10] and female[11] rat and, although hypertensive when acutely administered to the cat[12], corroborating the earlier work of Epstein et al.[2], it was shown to be antihypertensive when fed chronically to rats made hypertensive by successive injections of DOCA-saline[12]. There thus appeared to be a discrepancy in the results produced by PMPEA on blood pressure when administered chronically[12] as opposed to being injected acutely[2,3]. In order to resolve this discrepancy, a protocol was designed to study the potential effectiveness of daily administered PMPEA (in the food) in preventing and/or reversing chronic stress-induced hypertension.

Method. The method for inducing hypertension was that of Perach et al.[13], as modified by Segal et al.[14]. In summary, the method and experimental design was as follows.

30 adult male Sabra rats with an initial b.w of slightly less than 200 g, at the beginning of the experiment, were used in this study. The rats were divided into 3 groups of 10 (with no more than 4 rats being placed together in a cage) immediately upon their arrival in the laboratory, placed in the stress chamber and allowed to acclimatize for 11 days. Standard rat chow and water were allowed ad libitum.