increased at the rate of 0.47 W/kg °C. Animals which received 5,6-DHT, although showing no alterations in resting M, did show a higher thermosensitivity for the increase of heat production (0.84 W/kg °C) than the saline controls. According to Baumgarten et al.[9], 5,6-DHT destroys mainly 5-HT neurons after the compound infused directly into the brain tissue. In the present study, 5,6-DHT infused directly into the POAH in a corresponding dose produced an increase in both heat production and heat loss (as indicated by changes in whole peripheral circulation) at 8, 15 and 22 °C T_a. In these amine-depleted animals, the increased peripheral circulation counteracted the increased heat production, since rectal temperature remained constant. In fact, the results are consistent with a similar work of Lin and his co-worker[5] in rabbits. At first, one might consider the possibility that the increased heat loss caused by the peripheral circulation in animals depleted of hypothalamic 5-HT elicited a compensatory increase in metabolic heat production to maintain body temperature. The ability of these depleted animals to maintain a normal body temperature in the cold in the face of increased heat loss from the body surface, by increasing their metabolic heat production, raises a problem. Since internal temperature is normal and skin temperatures are equal to, or higher than normal (table), it is apparent that the thermal drive to increase heat production does not originate from the increased heat loss. Indeed, according to a recently proposed model for the regulation of metabolic heat production, it has been shown that an increased mean skin temperature tends to suppress, rather than increase, the heat production mechanism in the central nervous system (CNS)[12]. On the other hand, a more possible explanation might be found by postulating that 5-HT depletion in the CNS may produce an increased sensitivity in the metabolic heat production to thermal input (for example, cold stimuli). Apparently, the results observed for the hypothalamic administration of 5,6-DHT in rats are rather difficult to explain in terms of the current amine theory[13,14].

1 This work was supported by grants from National Science Council of Taiwan and J. Aron Charitable Foundation (USA). The author is grateful to Dr C.Y. Chai and Mr C.C. Wei for their support.
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Comparison of rat, mouse, guinea-pig and human slow reacting substance of anaphylaxis (SRS-A)[1]

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Summary. Slow reacting substance of anaphylaxis obtained from rat, mouse, guinea-pig and human tissues have exhibited similar biological activity and have reacted in the same way to chemical and enzymatic treatments. It is concluded that they appear to be the same substance or a similar class of compounds.

The presence of a biological material released from the anaphylactic lung, different from other known mediators and producing a contraction on the guinea-pig ileum with a slow onset (SRS), was first discovered by Kellaway and Trethewie[3]. This biological entity was later called 'slow reacting substance of anaphylaxis (SRS-A)' by Brocklehurst[4]. Although a lot of effort has been put into this field during the last 40 years, the biological significance and the chemical structure of this compound is still unknown and its importance as a mediator of hypersensitivity reactions is mostly based on indirect evidence.

Many groups have investigated the release of SRS-A from different species, including rat, rabbit, monkey, human and calf[5–7]. Being part of a larger project covering some aspect of SRS-A release during anaphylaxis and its significance in acute immunological reactions, the present investigation was carried out in order to compare some pharmacological, physicochemical and biochemical properties of rat, mouse, guinea-pig and human SRS-A.

Materials and methods. 1. Preparation of SRS-A. a) Guinea-pig SRS-A was prepared from the lungs of sensitized Dunking-Hartley animals according to Engineer et al.[8,9] and charcoal extracted[9,10]. b) Rat SRS-A was prepared from the peritoneal cavity by passive sensitization as described by Orange et al.[11]. c) Mouse SRS-A was obtained from the peritoneal cells of C3H mice treated with the ionophore A-23187[12]. The crude extract was then purified by ethanol extraction and Amberlite XAD-8 chromatography[13]. Some samples have been further purified by DE-52 ion exchange and silicic acid chromatography and have migrated as rat SRS-A characteristic pattern. d) Human SRS-A has been prepared from the supernatant of passively sensitized chopped human lung as described by Sirois et al.[14]. In some experiments, human SRS-A has been prepared according to Orange et al.[15,16] and ethanol extracted only.

2. Bioassay of SRS-A. SRS-A was measured on the guinea-pig ileum according to Engineer et al.[8], or to Orange and Austen[6].

3. Drugs used. The following drugs were used: mepyramine bimaleate (Poulenc; May and Baker); hyoscine hydrobromide (BDH Chemicals Ltd); arylsulfatase type V, α-chymotrypsin, lipoxidase and atropine (Sigma Chemicals Co.); elastase (Worthington Corp. and Sigma Chemicals

Slow reacting substance of anaphylaxis

Tests	Rat	Mouse	Guinea-pig	Human
1. Pharmacological				
a) Guinea-pig ileum	Contraction	Contraction	Contraction	Contraction
b) FPL-55712	Inhibition	Inhibition	Inhibition	Inhibition
c) release of RCS	ND	ND	+	+
2. Physico-chemical				
a) NaOH (0.05–0.1 N) 60 min, 100 °C	NI	NI	NI	NI
b) HCl (0.05–0.1 N) 60 min, 100 °C	I	I	I	I
3. Biochemical				
a) α-chymotrypsin	NI	NI	NI	NI
b) elastase	NI	NI	NI	NI
c) arylsulphatase	I	I*	I	I
d) lipoxidase	I	I	I	I

* Inactivated with human lung arylsulphatase supplied to us by Dr S.I. Wasserman. ND, not determined; NI, not inactivated; I, inactivated.

Co.); activated charcoal (Sutcliffe Speakman & Co., England); A-23187 and FPL-55712 KA were kindly supplied by Eli Lilly and Fisons Ltd respectively.

Results. 1. Pharmacological tests. The myotropic action of rat, mouse, guinea-pig and human SRS-A was first compared on the guinea-pig ileum strip. Following the injection of small quantities in the organ bath, each preparation of SRS-A elicited a slow contraction of the tissues which could be inhibited by FPL-55712[17]. Using our preparation of guinea-pig SRS-A, the potency of this inhibitor was tested in order to find the most effective concentration. For this purpose, series of guinea-pig ileum strips received by injection into the superfusing Tyrode solution, increasing amounts of SRS-A before and after the infusion of FPL-55712 for not less than 10 min over the tissues. In our system, 1 ng, 10 ng, 100 ng and 1 µg/ml of FPL-55712 in the superfusing fluid produced respectively 18.3%, 32.0%, 76.4% and 100% inhibition of the myotropic activity of guinea-pig SRS-A on the ileum strips (n = 15, 6, 9 and 12 respectively). Following SRS-A inhibition by FPL-55712, prostaglandin E_2 could still induce a contraction of the tissues.

The action of human and guinea-pig SRS-A was also studied on the release of 'rabbit aorta contracting substance' (RCS, a mixture of thromboxane A_2 and prostaglandin G_2 and H_2). The injection of both preparations of SRS-A into the pulmonary artery of a guinea-pig lung perfused with Tyrode solution elicited a strong contraction of the rabbit aorta strip which was perfused with the effluent, demonstrating the release of RCS activity.

2. Chemical tests. When rat, mouse, guinea-pig and human SRS-A were incubated for 60 min at 100 °C in the presence of HCl (0.05–0.1 N), a nearly complete loss of contracting activity on the guinea-pig ileum was observed in each case. On the other hand, the treatment of each preparation with NaOH (0.05–0.1 N) for the same period of time at the same temperature resulted in a good recovery of the myotropic activity of each preparation.

3. Biochemical tests. The incubation of rat, mouse, guinea-pig and human SRS-A in Tris buffer pH 8, or ammonium bicarbonate (1%) pH 8.5 with α-chymotrypsin (20–25 U/ml) at 37 °C for 60 min, resulted in a full recovery of the biological activity. Similarly, when another peptidic enzyme, elastase, was used at a concentration of 10 µg/ml in 1% ammonium bicarbonate buffer pH 8.5, different amounts of each preparation of SRS-A were perfectly resistant to digest (37 °C) for 60 min (in some experiments, we incubated for 12 h), indicating that SRS-A is not of peptidic origin. However, arylsulphatase (3.2 U/ml) in acetate buffer pH 5 destroyed each preparation of SRS-A to a large extent in 60 min at 37 °C. In this case, human lung arylsulphatase supplied to by Dr S.I. Wasserman was used to inactivate mouse SRS-A. Finally, a lipidic enzyme, lipoxidase (50–500 µg/ml) has been very effective to inactivate each preparation of SRS-A when incubated at 37 °C in Tyrode for 60 min.

Discussion. Our results with the inhibitor of SRS-A, FPL-55712, confirm the results obtained by Augstein et al.[17], since, in our conditions, this antagonist demonstrated a highly effective and dose-dependant blockade of the myotropic action of SRS-A on the guinea-pig ileum with an approximate ID_{50}-value of 25 ng/ml.

This work substantiates the observation that guinea-pig SRS-A releases RCS from the lung[18,19]; it shows that human SRS-A can also release RCS, indicating the similarity of both entities. Due to short supply, we have not tried the action of mouse and rat SRS-A on this model.

This report also shows by the use of chemical or biochemical tests, that rat, mouse, guinea-pig and human SRS-A are relatively stable to alkali, while unstable in acid solution. Enzymatic treatments of each preparation gave the same results; peptidic enzymes being without effect, while arylsulphatase and lipoxidase produced a high degree of digestion. This last enzyme is particularly interesting if we consider a) that it is involved in the oxidation of arachidonic acid, and b) that SRS-A has been recently[20,21] described as a metabolite of arachidonic acid. Experiments in progress will study more in detail this possibly important enzyme for the inactivation of SRS-A.

In conclusion, because rat, mouse, guinea-pig and human SRS-A have similar biological activities and because they react in the same way to chemical or enzymatic treatments, it appears that they could be the same substance or similar class of compounds. However, better purification[10] and eventually structure-elicidation are required to ascertain, on the basis of its chemical structure, the similarity between different preparations of SRS-A. For the present time, the high amounts of SRS-A released from different tissues and its potent biological activity are the most important proofs of its possible significance in acute immunological reactions in human as well as in other species.

1 Acknowledgments. The authors are grateful to Le Conseil de Recherche en Santé du Québec and to Fisons Ltd for financial support. This work was also partly supported by a grant to the late Dr R.P. Orange from the Medical Research Council of Canada (MT-4605).

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Precocene-induced collapse and resorption of corpora allata in nymphs of *Locusta migratoria*

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Summary. Precocene II causes an irreversible regression of corpora allata in young 4th instar nymphs of the migratory locust. Within 2 h after application, the cells collapse. Cell fragments are subsequently phagocytosed by haemocytes.

Precocene II (ageratochromene; 6,7-dimethoxy 2,2-dimethyl-chromene) is a compound isolated from the ornamental plant *Ageratum houstonianum*[2,3] which exhibits marked biological effects on several insect species. When administered to hemimetabolous larvae, a precocious metamorphosis follows, hence the name 'precocene'[3]. This effect closely resembles the effect of the extirpation of the corpora allata (CA). Indeed, there is some evidence, both physiological[3,4] and biochemical[5], that the CA cease to produce juvenile hormone (JH). In our laboratory, Pener et al.[6] found that the CA in morphogenetically abnormal, precocious 'adults' of *Locusta migratoria* which developed from precocene II-treated 4th instar nymphs, had atrophied. This raised the question whether the atrophy resulted from a direct toxic effect of precocene II on the glands, or from a more gradual process of regression due to lack of stimulation. In *Oncopeltus fasciatus,* signs of structural disintegration were found at 7 days after treatment[7]. We present here electron microscope data on CA of *L. migratoria* nymphs which indicate that precocene II acts in a matter of hours, possibly through a direct toxic action on the glands.

Material and methods. 4th instar nymphs of *Locusta migratoria migratorioides* R. & F., 16–24 h after the moult, were treated topically on the abdomen with 200 µg precocene II, dissolved in 2 µl acetone. Controls received only acetone. For incubation periods up till 18 h, the locusts were kept at 32–34 °C and 40–50% RH; for longer periods, in a climate chamber with a cyclically changing temperature and RH[6]. Locusts were sacrificed at intervals from 90 min to 15 days and corpora allata were dissected and processed for electron microscopy as described before[8].

Results. The ultrastructure of CA in normal *L. migratoria* nymphs has been described by Joly et al.[9]. Here, the observed deviations from the normal picture will be emphasized. Individuals showed some variability in response but the general pattern of structural changes is clear and can be summarized as follows.

In some of the glands fixed as early as 90 min after treatment, a condensation of the cytoplasm of most of the allatum cells occurred. The cytoplasm appeared more electron-dense due to a closer packing of ribosomes and other cytoplasmic constituents. The extracellular spaces which were relatively small in the controls (figure A) appeared enlarged at the same time (figure B).

After 2–3 h, the condensation process had proceeded in all individuals. The cytoplasm reached a high electron density, the chromatin in the nuclei became condensed as well, and the nuclear membrane lost its smoothness. The size of extracellular cisternae had further increased.

After 7 h the process of condensation resulted in a maximal shrinkage of the cells. Nuclei became small and of irregular shape, the chromatin coagulated and attained a very high electron density. The cytoplasm now reached maximal density, ribosomes were packed tightly in certain cytoplasmic areas (see also figure C). Mitochondria appeared shrunken and their membranes became vague. Microtubules remained intact and continued to support the cell's 3-dimensional shape; however, since little cytoplasm was left, the cells lost intimate cell to cell contact normally provided by desmosomes. Extracellular spaces had reached maximal dimensions. Preliminary observations indicate that the total volume of the gland had not markedly changed by this stage.

After 12 h, the structure of the gland cells had not changed much further. The nuclear membrane was not always discernable but occasionally a narrow perinuclear space was seen. The remains of the nucleus had a very irregular shape but fragments did not coalesce with the cytoplasm. The basement membrane surrounding the CA consisted of a loose texture of collagen filaments, and was interrupted at several places. Haemocytes, both granulocytes and plasmatocytes[10], penetrated and migrated deep in between the CA cells.

After 18 h, the haemocytes were quite numerous and had started engulfment and phagocytosis of cell fragments. The digestion of these fragments occurred very quickly. Details about the resorption process will be published elsewhere.

After 26 h, approximately 75% of total gland material had disappeared, probably by haemocyte action. The rather small condensed cell fragments that were left at this stage often contained a piece of nucleus. Cytoplasmic vacuoles of sizes up till 1 µm had developed; these contained a homogeneous, possibly lipoid, material of moderate electron density (figure C).

A small number of cells in the periphery of the gland responded in a different way to precocene II. These light cells contained clear and spheroid nuclei and did not condense as much as the predominate type of cell. Ultimately the nuclear chromatin became necrotic and pieces