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PHARMACOLOGICAL STUDIES OF 'SAPO' FROM THE FROG *PHYLLOMEDUSA BICOLOR* SKIN: A DRUG USED BY THE PERUVIAN MATSES INDIANS IN SHAMANIC HUNTING PRACTICES

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V. ERSPAMER, G. FALCONIERI ERSPAMER, C. SEVERINI, R. L. POTENZA, D. BARRA, G. MIGNOGNA and A. BIANCHI. Pharmacological studies of 'sapo' from the frog *Phyllomedusa bicolor* skin: a drug used by the Peruvian Matses Indians in shamanic hunting practices. *Toxicon* **31**, 1099–1111, 1993.—The dried skin secretion from *Phyllomedusa bicolor*, 'sapo', is used by the Matses Indians of the Northern Peru, in shamanic rites mainly designed to improve luck in hunting. When rubbed into burned, exposed areas of the skin, the drug causes the prompt appearance of violent peripheral gastrointestinal and cardiovascular effects soon followed by remarkable central effects (increase in physical strength, heightening of senses, resistance to hunger and thirst, exalted capacity to face stress situations). All the peripheral and most of the central effects of 'sapo' can be ascribed to the exceptionally high content of the drug (up to 7% of its weight) in potently active peptides, easily absorbed through the burned, inflamed areas of the skin. The concentration in 'sapo' of the single peptides (phyllocaerulein, phyllomedusin, phyllokinin, dermorphins and deltorphins) has been determined by bioassay, and peptide contents were correlated with the different symptoms of the 'sapo' intoxication.

INTRODUCTION

OVER the past few years we have had the opportunity to study the biogenic amines and particularly the active peptides in the skin of several hylid frogs from Central and South America belonging to the Phyllomedusinae subfamily (ROSEGHINI *et al.*, 1986; ERSPAMER *et al.*, 1986).

Particular attention was paid to *Phyllomedusa sauvagei* and *P. hypochondrialis* from Northern Argentina, *P. rohdei* and *P. burmeisteri* from Central Brazil and *P. bicolor* from the Peruvian and Brazilian Amazonas (ERSPAMER *et al.*, 1985).

We examined methanol extracts of both fresh and dried skins, with comparable results. This material did not contain appreciable amounts of amines (except 10–20 μg bufotenine per g dried skin in *P. rohdei*), nor bioactive alkaloids, but enormous amounts of a variety of active peptides belonging to at least seven families: caeruleins, tachykinins, bradykinins,

FIG. 1. A *Phyllomedusa bicolor* SPECIMEN, WITH DIVARICATED LEGS, BOUND TO FOUR SMALL STAKES. The frog is ready for collection of sapo (bamboo stick). Photograph by Peter Gorman, with permission (December 1990, Galvez River, near Angamos, Peru).

bombesins, sauvagine (corticotropin-releasing hormone-like peptide), tryptophyllins and opioid peptides, selective for either μ- or δ-receptors (dermorphins and deltorphins). The pharmacological actions of these peptides have been described in a number of papers (ERSPAMER and MELCHIORRI, 1983; ERSPAMER *et al.*, 1985).

In the course of our research on skin extracts of *P. bicolor*, our attention was attracted by a report by GORMAN (1990), dealing with some shamanic practices by the Matses Indians of the north-east corner of Peru, a subdivision of the Mayeruna tribe, in which the use of the drugs of animal and vegetable origin played a prominent role.

One of these drugs, 'sapo', was of particular interest because it consists of a dry, resinish substance obtained from a large tree frog which the Matses call 'dow kiet'. It is now well established that the frog is actually *P. bicolor* (cf. DALY *et al.*, 1992).

Sapo is employed in magic hunting rituals designed to improve the hunter's luck. To collect sapo the Matses catch the frog and keep it for 3 days during which time its legs and back are periodically and gently scraped with a stick of bamboo (Figs 1 and 2).

To dry the secretion collected from the frog, the stick is placed in a leaf bag and hung above the cooking fire between scrapings. The same stick is used over and over; by the end of 3 days the stick is covered with a yellow substance and the dow kiet is set free. The sapo retains its potency for at least a year (GORMAN, personal communication).

To use sapo, a Matses hunter first scrapes a bit of the dried resin from the stick, then mixes it with saliva until it has a consistence somewhat like mustard. The recipient's arm or chest is then burned with a smouldering twig, the burned skin is peeled away and the liquified sapo introduced into the raw area, approximately the size of a matchhead.

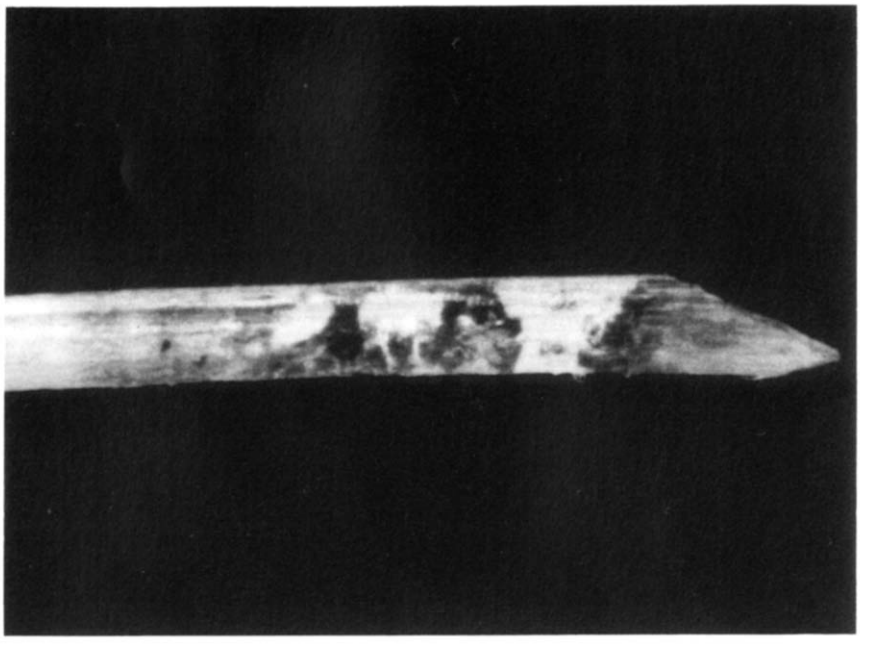

FIG. 2. BAMBOO STICK WITH THE SAPO MATERIAL USED IN THIS STUDY.

The effects elicited by the drug are described by GORMAN (1990), in a rather journalistic style, as follows:

"The effects are astounding: the moment the drug is placed upon your skin your body begins to heat up. In moments you feel as though you're burning from the inside; you begin to sweat. Your blood begins to race; your heart pounds. You became aware of every vein and artery in your body and feel them opening to allow for the fantastic pulse of your rushing blood. Your stomach cramps and you vomit violently. You lose the control of your bodily functions; you may urinate and defecate and drool uncontrollably. You fall to the ground and begin to lose consciousness; then suddenly, you may feel urges to do things you've never done before. You might find yourself growling, barking or moving about all fours. You feel as though animals are passing through you, trying to express themselves through your body. But even this extraordinary feeling is secondary to the speeding of your blood, a motion so fast that you think your heart would burst.

"For about fifteen minutes the rushing gets faster and faster, you are in agony. The pain becomes so great that you wish you could die and get it over with, but you don't die. The pounding slowly becomes steady and rhythmic and you gasp for air like a man saved from drowning. Finally the pounding subsides and you're overcome with exhaustion. You sleep.

"There are no dreams or visions with 'sapo'; you may even wonder what it all was for until you wake, then you are a god. Everything about you is larger than life, and your physical strength is explosive. You can do without food for several days and run in the jungle for hours without tiring. You can see in the dark effortlessly. You see animals before they see you, and you sense which plants are benevolent and which are not. Every

sense you possess is heightened and somehow in tune with the jungle, as though the 'sapo' put the rhythm of the jungle into your blood."

The violent visceral effects of sapo have been confirmed by GORMAN (personal communication) in about ten self-applications of the drug, and correspond exactly to the description by DOLE and CARNEIRO (quoted by FURST, 1974).

It is suggested that the drastic cleaning out of the body (vomiting, diarrhoea, urination, sweating) observed in the first phase of the sapo application, with alleged elimination of 'toxin', may have some 'magic' effect in itself and may heighten the effects of other drugs possibly taken prior to, or together with, sapo. Among other things, by cleansing the body, Matses hunters would lose their 'human' odour in the short time, making it easier to approach and capture the prey.

According to the estimates by GORMAN (personal communication) the amount of sapo rubbed into the normal two or three burned skin areas may be 20–30 mg (10 mg per area) with maximum doses of 100 mg per day. Sapo is used by the Matses not only to help on long hiking trips or in tapir trap setting (in this last case sapo is applied, morning and night, for at least 15 days, on four or five burns in the forearm and chest), but also to produce abortion (application on two burns to the interior of the vaginal labia).

With regard to the central 'magic' effects of sapo, things are perhaps more complicated. It is possible, and in some cases certain, that before or after sapo, the Matses (especially the Amahuaca) take other drugs such as 'Banisteropsis vine' or 'nu-nu' snuff, which may have more or less potent hallucinogenic effects. Thus it is difficult to decide how much of the ecstatic trance experience may be ascribed to the frog poison and how much to other drugs. It should be stressed, however, that Gorman has experienced central effects, "to feel like god", after application of sapo alone.

The purpose of the present study, using an original sample of sapo from the Matses, was to establish: (a) the actual amount of bioactive compounds in sapo, especially amines and peptides; (b) the effects of the single active constituents or of a cocktail of them on the cardiovascular system and viscera; and (c) the effects of the same constituents on the central nervous system.

The results of this study would help us attempt to explain the two successive phases, peripheral and central, of sapo intoxication.

MATERIALS AND METHODS

Sapo material

A sample of sapo was placed at our disposal, in February 1991, by PETER GORMAN. The skin secretion was obtained from a *P. bicolor* specimen weighing 72 g, captured by the Matses near the Galvez River, Peru, on 22 December 1990.

The material, resinous in aspect, weighed 126 mg. It was ground in a small mortar and then extracted with 30 ml of 80% methanol. After 2 days the supernatant was removed and extraction with a similar quantity of 80% methanol repeated twice, again for 2 days. The liquids were combined, filtered and kept at 2°C.

The exhausted sapo material, weighing 52 mg (41% of the total weight), was extracted first with 5 ml petroleum ether and then, after removal of the solvent, boiled in 3 ml of 0.1 M HCl, for 20 min. The methanol, petroleum ether and acid extracts (the last two colourless) were submitted separately to bioassay.

Chromatography on alumina

An amount of the methanol extract corresponding to 50 mg sapo was evaporated and the residue, weighing 36 mg, dissolved in 50 ml of 95% ethanol. The liquid was loaded onto a chromatographic column packed with 45 g of alkaline alumina (Merck, Darmstadt, F.R.G.) and eluted using a stepwise gradient of aqueous ethanol: 19 steps from 95 to 10% ethanol. At each step two or three fractions of 50 ml were collected. The eluates were kept at 2°C until used.

Thin layer chromatography and colour reactions

Samples of the sapo methanol extract corresponding to 0.5–2 mg drug were applied to silica gel plates and developed in 80% ethanol: acetic acid (10: 1 v/v). The developed chromatograms were then sprayed with Dragendorff reagent (for alkaloids) and the Ehrlich reagent, *p*-dimethylaminobenzaldehyde (for indolealkylamines).

Bioassay

The occurrence and concentrations of the various active components of the sapo extracts were determined using the following test preparations: caerulein-like peptides: guinea-pig gall bladder; bradykinin- and bombesin-like peptides: rat uterus; tachykinins: guinea-pig ileum and rabbit colon; dermorphins: electrically stimulated guinea-pig ileum; deltorphins: electrically stimulated mouse vas deferens; and sauvagine: rat diuresis.

All the above methods have been described in detail in previous papers (ERSPAMER and FALCONIERI ERSPAMER, 1962; ERSPAMER *et al.*, 1972, 1980; BROCCARDO *et al.*, 1981).

The guinea-pig gall bladder, rat uterus, mouse vas deferens and rat antidiuresis tests gave reliable qualitative and quantitative results even with the crude sapo extract. However, the quantitative evaluation of the tachykinins and dermorphins was possible only after separation by chromatography.

In a further set of experiments the crude methanol sapo extract as well as the petroleum ether extract were injected intracerebroventricularly in rats, in order to assess the direct central effects elicited by the drug.

RESULTS

Bioassay

(1) *Crude sapo methanol extract.* The content of active peptides was as follows: phyllocaerulein, expressed as caerulein (equiactive to the former) 30–35 μg per mg sapo; phyllokinin 18 μg/mg; sauvagine 3 μg/mg, finally, Ala-deltorphins, expressed as Ala-deltorphin I 5.3 μg/mg. Phyllomedusin and the dermorphins, as already stated, could not be determined in the crude extracts.

Intracerebroventricular injections in rats of the extract, in amounts corresponding to 20, 50, and 200 μg of drug per rat, produced only symptoms attributable to the opioid peptides: increased locomotor activity with rearing, grooming and sniffing, characteristic for the deltorphins (LONGONI *et al.*, 1991), and analgesia, characteristic for the dermorphins (BROCCARDO *et al.*, 1981). Threshold doses ranged from 20 to 50 μg; with 200 μg, effects were intense. No other obvious neurological or behavioural manifestations could be observed.

(2) *Petroleum ether extract.* The residue of the extract, taken up in water (slightly opalescent) did not display appreciable effects in any of our test preparations, up to amounts of 0.3 mg sapo/ml bath fluid. Similarly, the extract was completely inactive when given intracerebroventricularly in doses up to 3 mg sapo.

(3) *Acid extract.* This extract displayed $< 1\%$ of the activity of the methanol extract in our test preparations. Thus, only trace amounts of active compounds remained in sapo after methanol extraction.

(4) *Eluates from alumina column.* Figure 3 presents the elution profile of the various peptides from the alumina column. Sauvagine is retained by alumina and therefore appears only in trace amounts in the eluates. (a) Phyllocaerulein was eluted by 40 to 10% ethanol (26 μg/mg sapo), with a recovery of 80%; (b) phyllokinin appeared in 60_2 to 50_2 eluates (14 μg/mg), with a recovery of 78%; (c) phyllomedusin emerged in eluates 70_2 and 70_3 (18 μg/mg); assuming an 80% recovery (as inferred from other experiments) the amount occurring in the crude methanol extract would be 22 μg/mg; (d) the ala-deltorphins coeluted with phyllomedusin (4.6 μg/mg) with a 86% recovery (Fig. 4); (f) finally, [Lys^7]-dermorphin-OH was found in eluates 40 and 30 (0.2–0.25 μg/mg); assuming

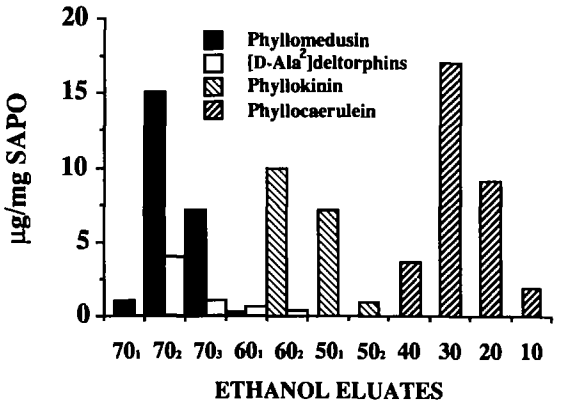

FIG. 3. ELUTION PROFILE FROM AN ALUMINA COLUMN OF THE FOUR MAIN PEPTIDES OCCURRING IN SAPO (IN µg PER mg SAPO).
Chromatography was obtained with a stepwise gradient of aqueous ethanol, from 95 to 10%. Numbers 1, 2, 3 (e.g. 70_1, 70_2, 70_3) refer to the 50 ml eluate fractions obtained successively at the same ethanol concentration. [Lys^7]-dermorphin-OH was eluted by 40 and 30% ethanol (not shown). Sauvagine was retained by alumina. Peptides were quantitatively estimated by bioassay, against their synthetic standards, using the smooth muscle preparations shown in Fig. 5.

a 70–80% recovery from alumina, the actual content of the peptide in sapo would be 0.25–0.33 µg/mg.

Methanol extracts of fresh *P. bicolor* skin also contain similar amounts of [Trp^4, Asn^7]-dermorphin-OH. The peptide could not be detected in the sapo extract probably because it was largely inactivated during drying of the cutaneous secretion. We have demonstrated (unpublished observations) that [Trp^4, Asn^7]-dermorphin-OH is attacked with exceptional rapidity by tissue endoproteases.

From data obtained in the bioassay of the crude sapo methanol extract and of the eluates from the alumina column, it can be calculated that active peptides account altogether for 7.2% of the sapo weight and for 12% of the weight of the methanol-extractable constituents of the drug.

Thin layer chromatography

No Dragendorff- or Ehrlich-positive spots appeared on chromatograms following application of 2 mg crude methanol extract, confirming the negative results obtained on paper chromatograms of *P. bicolor* skin extracts, using amounts of up to 0.2 g fresh skin (unpublished observations).

DISCUSSION AND CONCLUSIONS

Sapo, the dried secretion obtained from *P. bicolor* skin, contains enormous amounts of bioactive peptides, potently affecting the cardiovascular system and the visceral functions: up to 32 µg caerulein per mg sapo, 18 µg phyllokinin, 22 µg phyllomedusin and 3 µg sauvagine. In addition it contains 5.2 µg Ala-deltorphin (expressed as Ala-deltorphin I) and 0.25–0.33 µg [Lys^7]-dermorphin-OH. Sapo does not contain, like *P. bicolor* skin extracts, detectable amounts of biogenic amines (especially indolealkylamines) and

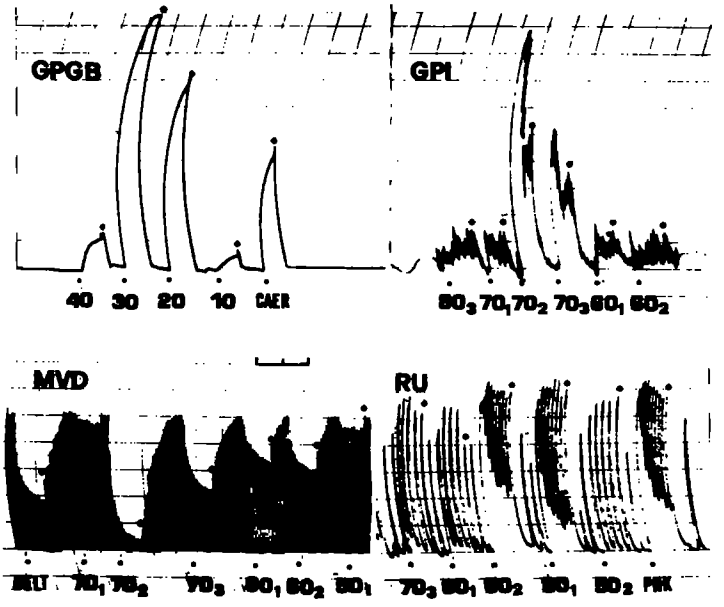

FIG. 4. SMOOTH MUSCLE PREPARATIONS USED IN THE QUALITATIVE AND QUANTITATIVE BIOASSAY OF THE DIFFERENT PEPTIDES OCCURRING IN THE ETHANOL ELUATES FROM AN ALUMINA COLUMN LOADED WITH SAPO.

Organ bath, 10 ml. GPGB, guinea-pig gall bladder. Responses elicited by amounts of eluates corresponding to 2 μg sapo. For comparison 10 ng caerulein (CAER). Peak of caerulein-like activity was found in 30% eluate. GPI, guinea-pig ileum. Responses elicited by amounts of eluates corresponding to 3 μg sapo. Peak of phyllomedusin activity was found in 70_2% eluate. MVD, mouse vas deferens. Response elicited by amounts of eluates corresponding to 10 μg sapo. For comparison 2 ng Ala-deltorphin I (DELT). Clear-cut peak of deltorphin activity was found in 70_2% eluate. RU, rat uterus. Responses elicited by amounts of eluates corresponding to 10 μg sapo. For comparison 100 ng phyllokinin (PHK). Peak of phyllokinin activity was found in 60_2 and 50_1% eluates. Time marks, 5 min.

Dragendorff-positive alkaloids. Other peptide peaks, in addition to those produced by the above peptides, are present in sapo HPLC chromatograms. Their study is in progress, but none of them displayed any activity in our systems.

At this point, before passing on to a detailed discussion of our results, a preliminary, fundamental question must be answered, that of the rate of absorption of the sapo constituents through the burned areas of human skin.

It is well known that exposed inflamed dermis (like inflamed mucosae) easily absorb drugs, as a consequence of vasodilatation and increased capillary permeability. Moreover, bradykinins and tachykinins, occurring in large amounts in sapo, are potent pro-inflammatory agents, further increasing vascular dilatation and permeability produced by the thermal injury.

Both bradykinin and eledoisin (a tachykinin related to phyllomedusin) are active, following intradermal injection in man, at doses as low as 1–5 ng (DE CARO, 1963).

As previously stated, it has been calculated that the sapo material applied to a burn has an approximate weight of 10 mg. If so, the peptide content of a single application would be as follows: caerulein 320 μg, phyllomedusin 220 μg, phyllokinin 180 μg, sauvagine 30 μg, Ala-deltorphins 53 μg and [Lys^7]-dermorphin-OH 3 μg.

On the basis of these qualitative and quantitative data we will try to explain both the peripheral and central symptoms observed in the two successive phases of sapo intoxication.

Peripheral aspects of the sapo intoxication

The single active peptides will be discussed in turn.

(a) *Caerulein* and the equiactive phyllocaerulein display a moderate action on blood pressure (threshold dose by s.c. injection in dog 5–10 μg/kg; in man possibly 1–2 μg/kg), but a potent action on the gastrointestinal smooth muscle, and gastric and pancreatic secretions (BERTACCINI *et al.*, 1968*a*, *b*; ANASTASI *et al.*, 1969; BERTACCINI, 1980). Intramuscular doses as low as 0.06 μg/kg produced in man relaxation of the sphincter of Oddi, with facilitation of bile flow, and doses of 0.5–1 μg/kg (threshold 0.1 μg/kg) provoked contraction of the gall bladder, potent stimulation of gastric and pancreatic secretions and potent stimulation of intestinal motility both in healthy subjects (AGOSTI *et al.*, 1970; BERTACCINI and AGOSTI, 1971) and in patients with post-operative intestinal atony (HAAS and FUEFF, 1978). Side-effects, observed with 1 μg/kg, were nausea, vomiting (3–10% cases), facial flush, mild tachycardia, changes in blood pressure, sweating, abdominal discomfort and urge for defecation.

Contraction of the gall bladder and increase in acid gastric secretion were observed also following nasal insufflation of 1 μg/kg caerulein (threshold 0.1 μg/kg) (AGOSTI and BERTACCINI, 1969). All the above effects and side-effects were obtained, in persons weighing 60 kg, at a total caerulein dose of 30–60 μg, an amount 15 to 20 times lower than the 640–690 μg of peptide present in 20–30 mg of sapo.

The obvious conclusion is that the gastrointestinal symptoms (vomiting, diarrhoea) observed in the early phase of sapo intoxication may be predominantly, if not entirely, an expression of caerulein intoxication. The peptide could also play a role in producing the cardiovascular effects elicited by the frog secretion.

(b) *Phyllokinin* is present in 20–30 mg sapo in amounts of 360–540 μg. Since the activity of this peptide on blood pressure in the dog is three times greater than that of bradykinin, the cardiovascular effects of the amount of phyllokinin occurring in sapo would be the same as those elicited by 1000–1500 μg bradykinin. In addition, the effect of phyllokinin on blood pressure lasts considerably longer than that of bradykinin, indicating a lower inactivation rate (ANASTASI *et al.*, 1966). No data are available on the amount of s.c. or i.m. phyllokinin or bradykinin necessary to cause a fall in blood pressure in dog or man. By i.v. injection 0.1 μg/kg phyllokinin is hypotensive in dogs and rabbits. Thus, it is possible that 5–7.5 μg/kg of s.c. phyllokinin may affect, at least transiently, blood pressure in man, contributing to the initial violent cardiovascular effects of sapo.

(c) *Phyllomedusin.* It is well known that this peptide (ANASTASI *et al.*, 1970), like all other tachykinins, is potently active on blood pressure, gut motility and lachrymal and salivary secretions (ERSPAMER and FALCONIERI ERSPAMER, 1962; BERTACCINI *et al.*, 1965; DE CARO *et al.*, 1966). The threshold hypotensive dose of the peptide in the dog, by s.c. injection, is 1–2 μg/kg; the stimulant action on gastrointestinal motility even lower: 0.2–1 μg/kg in the dog and probably also in man (BERTACCINI, 1980).

Thus, it is conceivable that the 440–660 μg phyllomedusin rubbed into the skin with 20–30 mg sapo (= 15–20 μg/kg) may substantially contribute to the cardiovascular effects of the drug and, even more, to its gastrointestinal effects.

(d) *Sauvagine.* The amount of this peptide present in 20–30 mg sapo is of the order of 60–90 μg. Peripherally, sauvagine causes in dogs, rabbits and rats a long-lasting fall in blood pressure, accompanied by intense tachycardia (ERSPAMER *et al.*, 1980). In the dog, pressure decrease is due mainly to an intense vasodilatation in the mesenteric vascular area. In rats, the threshold hypotensive and heart rate-stimulating dose of sauvagine, by s.c. injection, is 0.5 μg/kg (MELCHIORRI and NEGRI, 1983).

From the above data it seems likely that the amount of sauvagine rubbed into the skin with 20–30 mg sapo (corresponding to 1–1.5 μg/kg) may contribute to the cardiovascular effects ("blood racing, heart pounding") seen in sapo intoxication, as well as, through its potent vasodilator action on gut mucosal vessels, to diarrhoea. The effects of the activation of the pituitary–adrenal axis produced by sauvagine will be discussed later.

(e) *Opioid peptides.* No peripheral effects whatsoever can be expected from the amounts of Ala-deltorphins (105–160 μg) and [Lys^7]-dermorphin-OH (6–9 μg) occurring in 20–30 mg sapo.

In summary, it may be reasonably concluded that the intense peripheral cardiovascular and gastrointestinal symptoms observed in the early phase of sapo intoxication may be entirely ascribed to the known bioactive peptides occurring in large amounts in the frog material. A predominant role seems to be played by caerulein, with potentiation of the caerulein intoxication by phyllomedusin, phyllokinin and sauvagine. Abortion ascribed to sapo may be due either to direct effect of the peptide cocktail on the uterine smooth muscle or, more likely, to the intense pelvic vasodilatation and the general violent physical reaction to the drug.

Central aspects of the sapo intoxication

(a) *Caerulein*, given s.c. in rats, at a dose of 2 μg/kg, displayed clear-cut satiety and analgesic effects (JURNA and ZETLER, 1981). In man, an analgesic effect was observed, following s.c. administration of caerulein, at doses of 0.06–0.2 μg/kg (4–12 μg/60 kg) in patients suffering from renal colic, rest pain due to peripheral vascular insufficiency and even cancer pain (ERSPAMER, 1981, 1983; GRAIFF, 1990). It has been suggested that the analgesic effect, independent of any action on smooth muscles, may be related to the release of β-endorphin or to a direct effect of the peptide on the aminergic system in the CNS. On the other hand, caerulein given by i.v. infusion at a rate of 2 ng/kg/min (total 7 μg) caused a significant reduction in hunger and food intake in human volunteers (STACHER *et al.*, 1982).

The amount of caerulein rubbed into the skin with 20–30 mg sapo (11–16 μg/kg) would be largely sufficient to produce satiety and analgesic effects in man.

(b) *Sauvagine*, given to rats by the s.c. route, from threshold doses of 0.3 μg/kg, caused the release of corticotropin from the pituitary, with consequent activation of the pituitary–adrenal axis and an increase in plasma corticosterone levels. With 0.5 and 2 μg/kg, levels rose by 100% and 400%, respectively. The effects lasted for 2 to 4 hr. Simultaneously, there was a release of β-endorphin and an elevation of plasma catecholamine and glucose concentrations (VALE *et al.*, 1981; ERSPAMER and MELCHIORRI, 1983).

It should be remembered that according to GORMAN (personal communication), the maximum amount of sapo rubbed into the skin over the course of a day could be as high as 100 mg (corresponding to 3000 μg caerulein and 300 μg sauvagine) and that Matses hunters setting traps could apply 20–30 mg sapo to their skin twice daily for 10–15

consecutive days. It is clear that this would cause a persistent activation of the pituitary–adrenal axis by sauvagine and full manifestation of the analgesic and satiety effects due to caerulein.

(c) No appreciable central effects are to be expected by the amounts of phyllomedusin and phyllokinin present in 20–30 mg sapo, unless they increase the blood–brain barrier permeability, thus facilitating access to the brain not only of themselves but also of the other active peptides. This would perhaps allow the appearance of the anti-dipsogenic effect of phyllomedusin, with consequent enhanced resistance to thirst (DE CARO *et al.*, 1988).

(d) We do not know whether the Ala-deltorphins present in 20–30 mg sapo (120–180 μg) could have any central action in man, when administered by the s.c. route. It seems very improbable. In rats, intracerebroventricular doses as high as 0.1–0.5 μg/kg are required to elicit an increase in locomotor activity and other behavioural changes (ERSPAMER, 1992). Volunteers, infused with a total of 5 mg of Ala-deltorphin I over a 30 min period, showed no symptoms whatsoever (DEGLI UBERTI *et al.*, 1989). The same can be said for [Lys^7]-dermorphin-OH present in 20–30 mg sapo in an amount of 5–8 μg. In rats an analgesic effect could be appreciated only after 1–2 mg/kg of the peptide, given by s.c. injection.

In conclusion, some central effects of sapo (increase in physical strength, enhanced resistance to hunger and thirst and, more generally, increase in the capacity to face stress situations) may be explained by the presence of caerulein and sauvagine in the drug; the role played by the other peptides is uncertain. Of course, it cannot be ruled out that the cocktail of peptides occurring in sapo, owing to its effect on the vasculature, may increase the permeability of the blood–brain barrier, facilitating access of all peptides to the brain.

At any rate, no hallucinations, visions or 'magic' effects are produced by the known peptide components of sapo. Similarly, biogenic amines are beyond consideration, because *P. bicolor* skin, like that of other Phyllomedusinae, does not contain detectable amounts of these compounds.

In theory, it is not possible to exclude that other unknown sapo constituents, not detectable in our bioassay procedures, display some central effects in man. This, however, seems highly improbable because intracerebroventricular injections of sapo (methanol and petroleum ether extracts) in rats did not produce neurological or behavioural effects other than those expected from their content in known peptides, especially opioid peptides.

Recently, DALY *et al.* (1992) have isolated from a sample of *P. bicolor* skin secretion (sapo) a 33-residue linear peptide, very rich in alanine and lysine (11 and 6 residues, respectively) and possibly including in the sequence a D-amino acid residue:

Gly-Leu-Trp-Ser-Lys-Ile-Lys-Glu-Val-Gly-Lys-Glu-Ala-Ala-Lys-Ala-Ala-Ala-Lys-Ala-Ala-Gly-Lys-Ala-Ala-Leu-Gly-Ala-Val-Ser-Glu-Ala-Val-OH

The peptide, named adenoregulin, enhances binding of agonists to A_1-adenosine receptors in rat brain membranes. It is accompanied by peptides, as yet not sequenced, that inhibit binding. It is difficult to conceive that adenoregulin has any important participation in the peripheral and central effects of sapo. The enormous s.c. dose of 6 μg/mouse (approx. 240 μg/kg) caused only modest behavioural depression. The s.c. injection into mice of methanol or aqueous extracts of *P. bicolor* dried skin secretion in doses equivalent to 0.1–6 mg/mice of the dried material (= 4–240 mg/kg) resulted in a dose-related reduction of spontaneous locomotor activity. At higher doses, mice were completely inactive for several hours, although when touched they would respond briefly and then reassume a lethargic state. According to DALY *et al.* (1992) the inactive state is

reminiscent of the inactive but responsive state caused in mice by injection of some non-natural adenosine analogs.

Unfortunately no data are presented on the blood pressure situation of the injected mice. Reduction of locomotor activity and lethargic-like state, with preservation of responsiveness to stimuli are typical of severe hypotension, that may be produced by the enormous amounts of vasoactive peptides (up to 70 μg/mg) occurring in the dried skin secretion. The Matses share the 'magic' use of frog poisons with other Peruvian Indians, the Amahuaca (CARNEIRO, 1970) and the Cashinaua (KENNETT KENSINGER, quoted by FURST, 1974) as well as with the Brazilian Indians Mayoruna (cf. DALY *et al.*, 1992) and Marubo (MONTAGNER-MELATTI, 1985). The Cashinaua seem to employ a different frog species, and to interpret the experience as one of purification, designed to expel a sickness-like condition and bad luck, especially in hunting.

ROTH (1915) has reported similar 'magic' practices among the Indians of the Guineas. The poisonous exsudation and spaw of certain frogs or toads are rubbed into cuts made in the skin, or introduced into the eyes, nose, mouth and ears of the hunters, with the same drastic symptoms experienced during sapo intoxication.

We have studied as many as 12 Phyllomedusinae frogs collected from Northern Argentina to Mexico, and have shown that all the examined species contained the same cocktail of active peptides (bradykinins, tachykinins, caeruleins, sauvagine, opioid peptides) as *P. bicolor* skin (ERSPAMER *et al.*, 1985). Hence, the skin secretion of all these frogs could provoke an intoxication similar to that elicited by sapo. Several *Phyllomedusa* species, e.g. *P. tomoptera*, *P. palliata* and *P. tarsius*, are sympatrid to *P. bicolor*. In accordance with this assumption, the injection of a methanol extract from a skin of *P. lemur*, that was equivalent to 100 mg of wet skin, produced in mice an inactive state similar to that caused by extracts of dry *P. bicolor* secretion (DALY *et al.*, 1992).

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