site. Since the lipid asymmetry seems to be a result of differential binding of phospholipids by proteins and other ligands on the two sides of the membrane (*18*), alterations in molecular organization of the lipid bilayer could induce changes in distribution of other membrane components. The suggested aggregation of the integral proteins of the host cell membrane during *P. knowlesi* infection (*5*) could therefore be a result of altered phospholipid asymmetry. The complete hydrolysis of the external PE in parasitized cells by phospholipase A_2 also suggests that the protein distribution in the host cell membrane is altered.

It is important to consider how the observed changes in composition and inside-outside distribution of phospholipids in the infected host cell membrane come about. We suggest that the parasite may secrete phospholipid-rich vesicles on the host cell surface at the time of its entry into the cell. These vesicles may then fuse with the host erythrocyte membrane causing changes in composition and assembly of phospholipids. The presence of lipid vesicle-like structures on the host cell surface has already been demonstrated at the time of the merozoite's entry into the cell (*5*, *19*).

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Substance P Activity in the Bullfrog Retina: Localization and Identification in Several Vertebrate Species

Abstract. *Immunoreactive substance P is present in the bullfrog retina, possibly in two types of stratified amacrine cells, with their somas in the inner nuclear layer and their neuronal processes entering the inner plexiform layer and ramifying in sublayers 3 or 4 (or both). Occasionally, polygonal somas positive for substance P were found in the ganglion cell layer. Approximately 75 percent of the cell bodies positive for substance P and 65 percent of the radioimmunoassayable substance P were found in the superior half of the frog retina. On the basis of high-performance liquid chromatography, the immunoreactive substance P in the neural retina of the rat, monkey, or chick is similar to synthetic substance P, whereas this is not true of the immunoreactive substance P in the bullfrog or carp retina.*

Bioassayable substance P (SP) (*1*), now fully characterized as an undecapeptide (*2*), has been known to be present in the mammalian neural retina for over two decades. Early radioimmunoassay (RIA) results (*3*) of retinal material for SP are largely consistent with previous bioassay results; and the use of immunocytochemical (ICC) methods indicates that immunoreactive SP (IR-SP) is present in at least one type of amacrine cell in the pigeon retina (*4*). Consistent with the knowledge that IR-SP is present in neuronal cell types of certain vertebrate retina are our findings that intravitreous injections of kainic acid in bullfrogs or rats results in a dramatic reduction of several peptides in the neural retina, including IR-SP (*5*). Furthermore, the hypothesis of a neurotransmitter function for retinal peptides is supported by the observation that depolarizing concentrations of K^+ induce the release of retinal thyrotropin-releasing factor (TRH), somatostatin, and SP in vitro from bullfrog retinas in a Ca^{2+}-dependent manner (*5*). The concentration of IR-SP in the neural retina was higher in the bullfrog than in any other vertebrate species examined. We therefore determined the type and regional localization of IR-SP in the bullfrog retina and obtained additional clarification of the SP activity in retinal extracts of

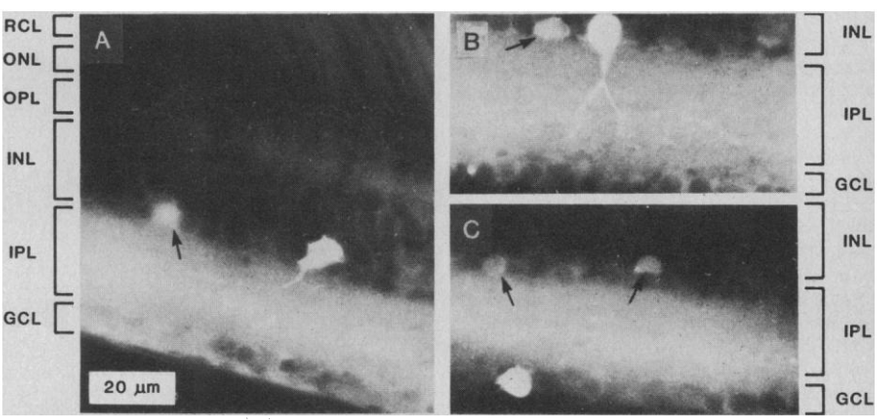

Fig. 1. Demonstration of immunoreactive substance P in neuronal elements of horizontal sections (10 to 30 μm) of the bullfrog (*Rana catesbeiana*) retina. (A) Polygonal amacrine cell with perikaryon in the inner portion of the inner nuclear layer (*INL*) and neuronal processes entering the inner plexiform layer (*IPL*). (B) Pyriform amacrine cell with cell body in the inner nuclear layer and neuronal processes ramifying in the inner plexiform layer. (C) Perikaryon of displaced amacrine cell in the ganglion cell layer (*GCL*). Arrows indicate the presence of additional SP-positive somas that are out of the plane of focus. *OPL*, outer plexiform layer; *ONL*, outer nuclear layer; *RCL*, receptor cell layer.

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frog, monkey, rat, and bullfrog by reverse-phase high-performance liquid chromatography (RP-HPLC).

Adult bullfrogs (600 to 800 g) received an intravitreous injection of colchicine (50 μg per 10 μl in 0.9 percent saline) 16 hours before fixation. Routine tissue preparation and immunohistochemical staining (indirect immunofluorescence) procedures were followed (*6*). Antiserum to SP (SK-SP1) was used at dilutions of 1:500 to 1:1000 for the ICC localization of SP; for the RIA determination of SP, the antiserum was used at a final dilution of $1:5 \times 10^6$ (*5*). Preliminary absorption of the SP antiserum with $10^{-6}M$ SP eliminated any specific staining of somata or neuronal processes in the retinal sections.

By indirect immunofluorescence procedures, SP-positive perikarya or stratified amacrine cells were found in the most proximal area of the inner nuclear layer (Fig. 1, A and B); with far less frequency, SP-positive cell bodies were found in the ganglion cell layer (Fig. 1C). In general the morphology of the stratified amacrine cells revealed either a pyriform (Fig. 1B) or polygonal cell body, and sometimes short spines could be seen emerging from the polygonal soma (Fig. 1A). The major process of each cell projected proximally to the inner plexiform layer and generally divided into two or more branches before ramifying in sublayer 3 or 4 (or both) of the inner plexiform layer. The cell bodies in the inner nuclear layer were 7 to 9 μm in diameter and characteristically had a thin rim of immunoreactive cytoplasm surrounding a nonreactive nucleus that filled the cell body. There was often an accumulation of finely granular immunoreactive material in the initial segment of the major process. After the initial branching, varicose fibers with few branches usually ramify in a single sublayer (sublayer 3 or 4) of the inner nuclear layer; in tangential sections through the inner plexiform layer, these varicose fibers could be traced for several millimeters. The SP-positive amacrine cells are similar to type L and N amacrine cells (*7*). Although SP-positive cell bodies occur in the ganglion cell layer (Fig. 1C), we were unable to visualize any fibers entering the optic nerve; therefore these cells are probably displaced amacrine cells. The superior region of the neural retina contained more SP-positive perikarya than the inferior retina did, and the inferior nasal quadrant contained the least of all. The significance of this unequal distribution of cell bodies is not apparent at this time.

Results obtained with radioimmunological methods indicate that a substance resembling SP is present in the neural retina of several vertebrate species. Since molecular heterogeneity of a particular peptide occurs in different species, and multiple forms of a single peptide exist in different regions within a given species (*8*), we analyzed retinal extracts of several vertebrate species by RP-HPLC. Overall, several peaks of SP activity could be resolved under isocratic elution conditions (Fig. 2). All of the IR-SP in the frog differed from synthetic SP, whereas all of the IR-SP present in an extract of rat or monkey retinal tissue had a retention time identical to that of authentic SP. In addition, most of the IR-SP in chicken retinal material eluted at a retention time similar to that for authentic SP. The IR-SP in carp (*Cyprinus carpio*) retina appears to be identical to that in the bullfrog retina, on the basis of HPLC results (*9*). Three distinct IR-SP substances in the bullfrog retina (or carp retina) can be separated from authentic SP by gradient-elution HPLC, although this is not apparent under isocratic elution conditions (Fig. 2).

In the neural retina of higher vertebrates (monkey and rat, for example), the IR-SP appears to be authentic SP, whereas in the retina of lower vertebrates, a significant amount (chick) or all (frog and carp) of the IR-SP is different from synthetic SP. Since several SP-like polypeptides of nonmammalian origin are known (*10*), including physalaemin, phyllomedusin, uperolein, eledoisin, and kassinin, it is imperative to know if one or more of this group can account for the SP-like substances in the bullfrog and carp. Although antiserum to SP has a 100 percent cross-reaction with physalaemin or uperolein, HPLC (Fig. 2) indicates that physalaemin and uperolein are not the SP-like peptide in the neural retina of the bullfrog or carp (*9*). Kassinin, eledoisin, and phyllomedusin are not likely candidates because of their lack of cross-reaction with the antiserum (*5*, *9*). On the basis of the results of RIA and HPLC, it appears that the SP-like substances in the bullfrog or carp retina represent novel peptide moieties.

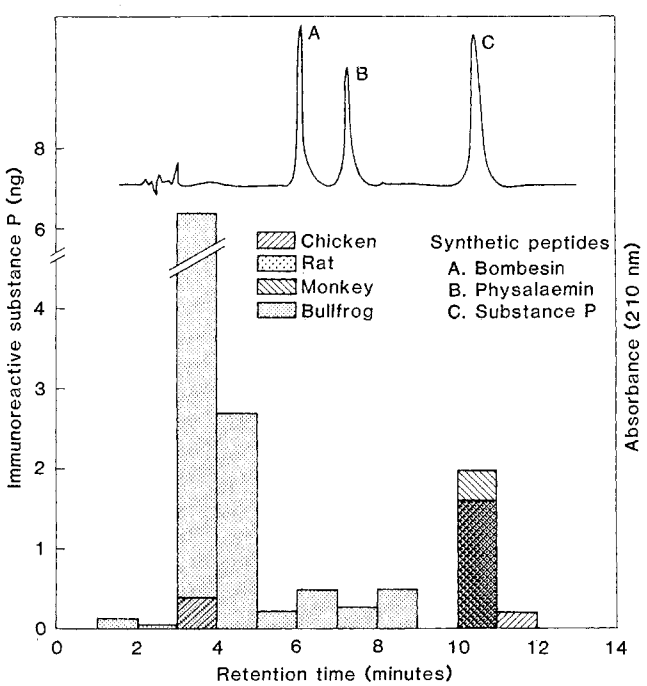

Fig. 2. Partial characterization by HPLC of IR-SP present in retinal extracts of chicken (5-day-old Dekalb Leghorn), rat (Osborne-Mendel, National Institutes of Health), monkey (*Macaca irus*), and bullfrog (*Rana catesbeiana*). Retinal tissue was processed after enucleation (*5*). Pooled retinal samples were purified on a Sep-Pak C_{18} cartridge (Waters Associates); SP-like peptides were eluted from the Sep-Paks with 4 ml of 75 percent ethanol in 0.5 percent hydrochloric acid. The acid-ethanol eluant was dried under N_2 at 55°C, resuspended in distilled water, and clarified by centrifugation. Portions of the supernatant fluid or standard solutions were injected onto a Supelco C_{18} (0.46 by 15 cm) reverse-phase column (Supelco, Bellefonte, Pennsylvania). The column was eluted under isocratic conditions with triethylamine phosphate (78 percent) and acetonitrile (22 percent), *p*H 2.25. The flow rate was 1 ml/min and 1-ml fractions were collected and concentrated to dryness in a vacuum centrifuge. The IR-SP was determined by RIA (*5*). Recovery of synthetic SP or SP-like activity ranged from 75 to 92 percent throughout the Sep-Pak step and HPLC step.

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Phencyclidine, Lysergic Acid Diethylamide, and Mescaline: Cerebral Artery Spasms and Hallucinogenic Activity

Abstract. *Phencyclidine (PCP), lysergic acid diethylamide (LSD), and mescaline produced potent contractile responses on isolated basilar and middle cerebral arteries, where, in terms of potency, LSD > mescaline > PCP. All three drugs produced cerebrovasospasm in a concentration range which parallels that needed for their psychotomimetic and intoxicating actions. Specific receptors for PCP, which subserve contraction and differ from those for LSD and mescaline, are found in cerebral arteries. Concentrations of PCP that produced near-maximum contractile responses on cerebral arteries were similar to those in the blood and brain of human subjects who had died from PCP overdoses. A specific calcium antagonist, verapamil, readily prevented (and reversed) PCP-induced vasospasm. This study provides direct evidence for PCP receptors in cerebral blood vessels, the biologic action of which can be reversed by a calcium antagonist; the clinical use of the latter could prove invaluable in treating PCP-intoxicated victims.*

Phencyclidine hydrochloride intoxication, especially among young people, is reaching alarming and epidemic proportions (*1, 2*). Abuse of phencyclidine hydrochloride, often referred to as "PCP," "angel dust," or "hog," frequently appears to result in violent behavior and mortality among its users. Systemic administration of PCP, like lysergic acid diethylamide (LSD) and mescaline, has a profound effect on mental status, causing, for example, disorientation, psychosis, uncontrolled violent reactions, and convulsions; some individuals may experience hallucinations and a condition that has been linked to schizophrenia (*1, 2*). High doses of these drugs often lead to severe hypertension, a toxic acute brain syndrome (manifested by disorientation), a clouding of consciousness, and convulsions (*3, 4*). Mortality following ingestion of PCP, LSD, or mescaline is thought to be a result of cardiovascular or respiratory failure, the mechanisms of which are not clear (*3, 4*).

Since cerebral hypoxia has been suggested as playing a role in the psychotomimetic and lethal actions of PCP, LSD, and mescaline (*1–5*), we wondered whether these hallucinogenic drugs could exert direct actions on cerebral blood vessels. We report here that PCP, LSD, and mescaline produce vasospasm of isolated cerebral arteries; the ranges of the effective contractile concentrations parallel the psychotomimetic, intoxicating, and lethal concentrations of all three hallucinogenic drugs. The cerebral contractile effects of PCP could be completely abrogated (or prevented) by use of a calcium antagonist (verapamil) but not by any known specific pharmacologic antagonist.

Mongrel dogs of either sex weighing 15 to 20 kg were anesthetized with pentobarbital sodium (30 mg/kg). After thoracotomy, the brains were rapidly removed and the basilar and middle cerebral arteries were excised. Helical strips were cut from segments of these cerebral arteries; the strips were 10 to 15 mm long by 1.5 to 2.0 mm wide (*6*). The strips were suspended isometrically under 1 g of tension and incubated in 20-ml muscle chambers containing normal Krebs-Ringer bicarbonate solution (composition in millimoles per liter: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 10; and $NaHCO_3$, 25) at 37°C, through which a mixture of O_2 (95 percent) and CO_2 (5 percent) was bubbled (*6*). The force of the contractions was measured with Grass FT-03 force-displacement transducers and recorded on a Grass model 7 polygraph. Three hours after being incubated under tension, the preparations were exposed to KCl, serotonin (5HT), PCP, LSD, and mescaline. The KCl and 5HT were used as stimulants so that we could assess the vasoactive effects of the psychotomimetic drugs.

Addition of LSD or mescaline to the muscle chambers resulted in rapid, increased tension development in all cerebral arteries tested. This effect was followed shortly by a falloff in tension to a lower plateau, very similar to that observed for 5HT and KCl (Fig. 1). Addition of PCP resulted in rapid, increased tension development which was long-lasting and did not decrease in magnitude with time (Fig. 1). Cumulative addition of the psychotomimetic drugs to the cerebral vessels revealed that a relative order of contractile potency (based on threshold and half-maximum concentrations) can be obtained, where LSD > mescaline > PCP (Table 1 and Fig. 2). A comparison of these contractile concentrations to those needed for psychotomimetic and intoxicating actions in man not only reveals a parallelism (*1–4, 7*), but also demonstrates that the relative order of potencies, that is, LSD > mescaline > PCP, are also similar. Possibly more important was the finding that the concentrations of PCP that yielded near-maximum contractions on the canine middle cerebral and basilar

Table 1. Relative contractile sensitivity of canine basilar and middle cerebral arteries to PCP, LSD, mescaline, 5HT, and KCl. The values are given as means ± standard errors of the means. Minimum effective concentration is the concentration which produces a threshold contractile response. The EC_{50} is the concentration that produces 50 percent of the maximum contractile response.

Drug agonist	*N*	Minimum effective concentration (*M*)	EC_{50} (*M*)	Maximum tension (mg)
		Middle cerebral arteries		
LSD	6	$1.62 \pm 0.35 \times 10^{-9}$	$2.35 \pm 0.38 \times 10^{-8}$	466 ± 65.6
Mescaline	4	$5.15 \pm 1.22 \times 10^{-7}$	$3.75 \pm 0.65 \times 10^{-6}$	516 ± 78.4
PCP	9	$1.58 \pm 0.46 \times 10^{-7}$	$3.42 \pm 0.28 \times 10^{-5}$	676 ± 51.5
5HT	7	$4.97 \pm 0.52 \times 10^{-10}$	$1.42 \pm 0.16 \times 10^{-8}$	520 ± 73.8
KCl	4	$2.24 \pm 0.24 \times 10^{-3}$	$1.24 \pm 0.16 \times 10^{-2}$	700 ± 35.4
		Basilar cerebral arteries		
LSD	6	$2.56 \pm 0.68 \times 10^{-9}$	$2.92 \pm 0.46 \times 10^{-8}$	488 ± 58.4
Mescaline	6	$1.76 \pm 0.36 \times 10^{-7}$	$6.60 \pm 0.82 \times 10^{-6}$	720 ± 52.5
PCP	9	$1.84 \pm 0.52 \times 10^{-7}$	$4.62 \pm 0.44 \times 10^{-5}$	1400 ± 102
5HT	7	$1.97 \pm 0.84 \times 10^{-9}$	$5.88 \pm 0.76 \times 10^{-8}$	1293 ± 184
KCl	4	$4.20 \pm 0.65 \times 10^{-3}$	$1.68 \pm 0.32 \times 10^{-2}$	1325 ± 35.3

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