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Vasopressin-Like Effects of Psychotropic Drugs in Amphibian Epithelia

A. Grosso and R.C. de Sousa

Departments of Physiology and Medicine, School of Medicine, University of Geneva, 1211 Geneva 4, Switzerland

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Summary. Amphibian epithelia have been used as models for studying the effects of psychotropic drugs on membrane transport. Several of these agents added to the internal or to the external media, at concentrations greater than 10^{-3} M, had inhibitory, "ouabain-like" effects on Na transport. In contrast, stimulatory, "vasopressin-like" effects were seen at lower concentrations. The stimulation was additive to that of oxytocin if the drug was present in the external solution but nonadditive if in the internal solution. On water transport, harmala alkaloids had a vasopressinomimetic action in toad skin, while inhibition was seen with Li and amitriptyline. To account for these multiple effects, it is hypothesized that psychotropic drugs act on the following cell targets: the Na pump, the cyclic nucleotide system, microtubules, and membrane calcium sites at the outer barrier of the epithelium. Direct, biochemical evidence is needed to substantiate this hypothesis.

Despite intensive investigation for many years with a number of experimental models and techniques, the mode of action of psychotropic drugs remains largely unknown. Available evidence has shown, however, that these drugs can interact with two major components of the cell machinery: the Na pump and the cyclic nucleotide system [25, 45, 53]. These effects, as well as those reported on microtubules [22, 24, 41] and membrane calcium [46], strongly suggest that psychotropic drugs induce a variety of alterations in membrane transport mechanisms.

The study of transport processes is particularly difficult in vertebrate nerve cells, in view of the structural and functional complexity of the central nervous system. Consequently, it is useful to work with simpler systems in the hope of gaining insight into mechanisms that might be involved in the mode of action of psychotropic drugs. Amphibian epithelia have been widely used in the past 25 years to investigate the modulation of membrane permeability by hormones and drugs [9, 52]. Current knowledge on transport processes in these tissues indicates that they are ideally suited to the study of the effects of agents supposed to act

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on cyclic nucleotides and active Na transport. Besides, in the light of some more recent work [9], amphibian epithelia may also be considered as potentially useful models for examining the interplay between transport processes and the reported effects of psychotropic drugs on microtubules and membrane calcium.

Preliminary experiments with amitriptyline, performed in our laboratory five years ago, did show that this antidepressant drug affects Na transport across frog skin [9]. Subsequently, more elaborate studies with a hallucinogenic alkaloid, harmaline, confirmed and extended these observations [10, 13]. In this report, effects of psychotropic drugs in amphibian epithelia are reviewed, with particular emphasis on "vasopressin-like" effects on transport of Na and water.

Psychotropic Drugs and the Na Pump

The concept of Na pump, advanced by Dean in 1942 [8], gained new momentum in 1957 with the discovery by Skou [49] of a ATPase activity that was stimulated by Na and K in microsomal preparations from crab nerve. Subsequent work established that this enzyme system is a universal component of the plasma membrane of animal cells and very likely corresponds to one of the earliest functions of the membrane machine to evolve [54]. From the very beginning, cardiac glycosides were identified as inhibitors of (Na+K)-ATPase [45]. Along the years, however, many other substances, including psychotropic drugs, were found to share this property [45]. Chlorpromazine [39, 43, 45] and harmala alkaloids [4] have been particularly well studied.

In 1972, Medzihradsky and Nandhasri [34] reported that the tricyclic antidepressant amitriptyline was a potent inhibitor not only of brain (Na-K)-ATPase but also of the corresponding enzyme obtained from kidney cortex. By this time we were examining the effects of amitriptyline on another epithelium – the frog skin – and found ouabain-like effects on short circuit current (SCC) when this drug was added to the Ringer's solution bathing the internal surface of the skin of *Rana ridibunda*. In contrast to ouabain, however, addition of amitriptyline to the external solution produced a similar depression of SCC (Fig. 1).

The concentrations of amitriptyline used in these frog skin studies were similar to those needed to inhibit kidney ATPase [11, 34]. At 10^{-3} M, the antidepressant consistently brought SCC to near zero values, although in some instances a clear inhibition of SCC was already seen at

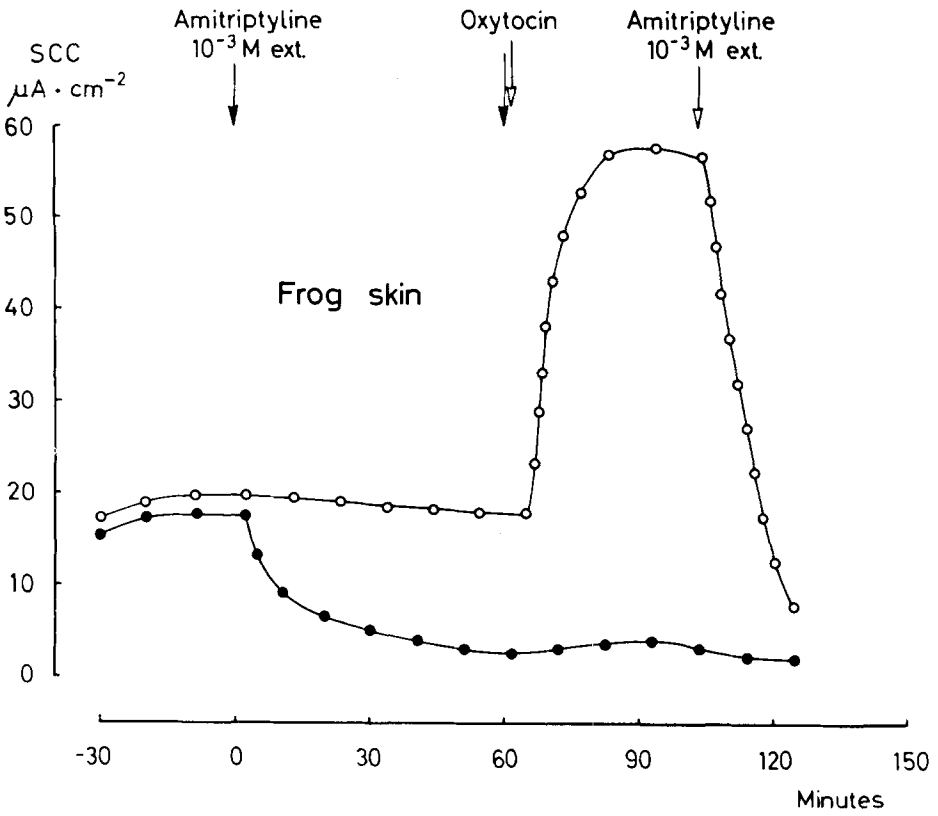

Fig. 1. Ouabain-like inhibition of SCC by amitriptyline added to the external solution bathing frog skin. Note the block of the hormone effect and the rapid decrease in SCC induced by amitriptyline after pre-exposure to oxytocin. In this and in the following figures the symbols ○—○—○ and ●—●—● indicate the SCC curves of two pieces of the same skin mounted in a double chamber

10^{-5}–10^{-4} M amitriptyline. Inspection of Fig. 1 reveals several typical features of the action of amitriptyline on frog skin. Both basal and oxytocin-stimulated SCC were depressed by the drug, but the time course was faster in tissues pre-exposed to oxytocin. In addition, oxytocin was ineffective in tissues pre-exposed to amitriptyline. The effect of amitriptyline was not always monophasic, as shown in Fig. 1. Quite often the decline in SCC was preceded by a transient, although conspicuous, rise in current.

In 1973, Canessa *et al.* [4] published a remarkable study on harmala alkaloids and the (Na+K)-ATPase system which prompted us to apply these hallucinogenic drugs to amphibian epithelia. As reported in detail elsewhere [13], harmaline and some of its analogues, at concentrations

varying from 1 to 5 mM, altered SCC in frog skin in a manner pratically identical to that previously seen with amitriptyline. Both types of drugs induced a faster inhibition of SCC in skins pre-activated with neurohypophyseal hormones or norepinephrine. Such acceleration of SCC inhibition, when the psychotropic agents were added to the external solution, might reflect a facilitation of drug permeation across the outer barrier of the epithelium. A similar phenomenon has been reported by Levine *et al.* [29] in toad bladders exposed to vasopressin. These authors did find an increase in permeability of the apical membrane to a variety of organic molecules. Widespread increases in membrane fluidity, mediated by cAMP, may be the cause of these permeability changes in both epithelia.

Amitriptyline and harmaline were both ineffective in skins pre-exposed to maximal concentrations of ouabain while they further depressed SCC in tissues pre-exposed to lower concentrations of the glycoside. Work from other laboratories also showed that harmala alkaloids [4, 15, 32], reserpine [32], chlorpromazine [30] and morphine [20] inhibit SCC in amphibian skin and bladder. The evidence obtained so far is compatible with, but does not prove, an inhibition of the Na pump of amphibian epithelia. This point must be clarified by direct measurement of the (Na+K)-ATPase activity in epithelia exposed to these psychotropic drugs. As far as the mechanism of inhibition is concerned, an intracellular site of action is likely, in view of the effectiveness of these drugs from both sides of the epithelia [13, 20, 30]. In this regard, harmaline stirred a great deal of interest as it appeared to be a fluorescent probe of the Na (intracellular) site of (Na+K)-ATPase. Recent work has challenged the specificity of this interaction [5, 42]. In any event, psychotropic drugs, particularly harmala alkaloids, remain promising tools for the chemical dissection of the Na pump.

Psychotropic Drugs and Cyclic Nucleotides

The ubiquitous cyclic nucleotide system of Sutherland possesses particularly high activities of adenylyl cyclase and cyclic nucleotide phosphodiesterase in brain [3, 37, 50, 53], but its role in the function of the central nervous system is far from being understood. Cyclic AMP has been implicated in aberrant behavior and in the mechanism of action of psychotropic drugs [25, 37, 38, 53]. More recently, tolerance to and dependence upon opiates and morphinomimetic peptides, have been rela-

ted to changes in adenylyl cyclase activity [27, 47, 48]. With this background information in mind, we looked for evidence of an interplay between psychotropic drugs and cyclic AMP in amphibian epithelia.

It could be anticipated that, if a drug raises cyclic AMP in frog skin, there should be a stimulation of Na transport nonadditive to that of oxytocin or norepinephrine. This prediction was apparently verified with harmaline. When the internal surface of the skin of frogs *Rana ridibunda* or toads *Bufo bufo* was exposed to concentrations of harmaline

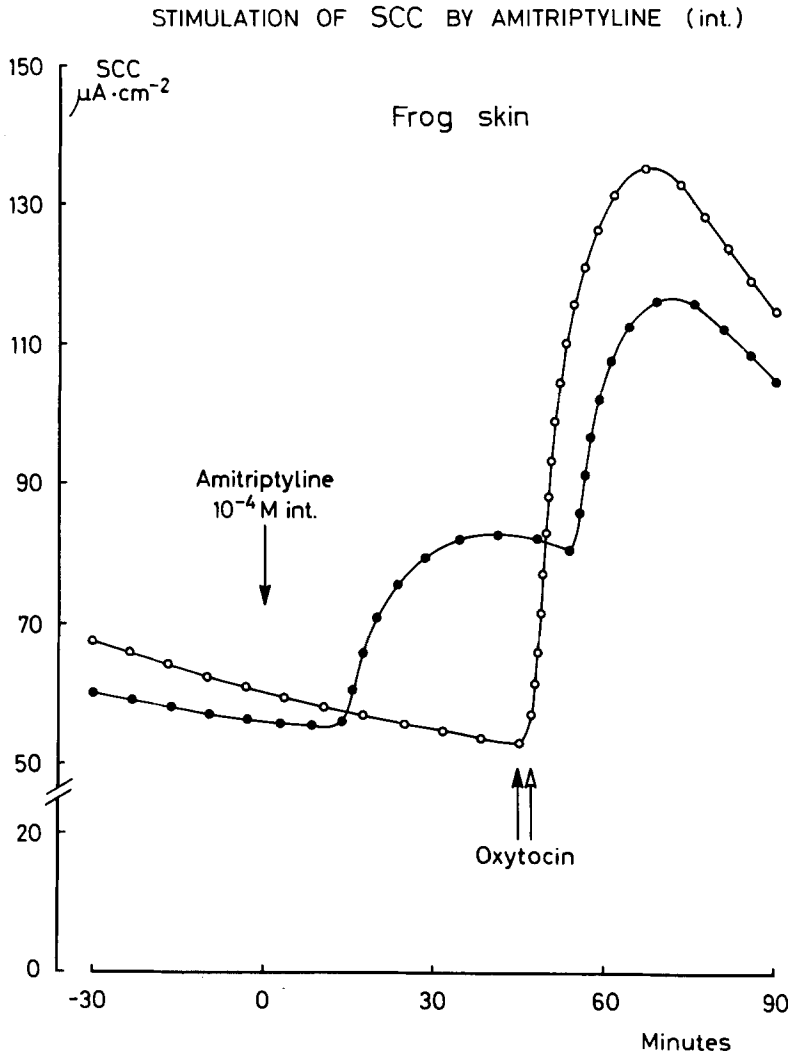

Fig. 2. Sustained stimulation of SCC by amitriptyline added to internal solution at a relatively low concentration. The effect is nonadditive to that of oxytocin given at a supramaximal concentration (50 mU/ml)

lower than those reported in the previous section, a marked stimulation of SCC was observed, as well as mutual inhibition between the hallucinogen and oxytocin [13]. Going back to amitriptyline we found that this, too, had a similar vasopressin-like effect [11] as shown in Fig. 2. Sensitivity of the skins to both drugs varied considerably and, in the range of $5 \cdot 10^{-4}$ M to 10^{-3} M, stimulation or inhibition of Na transport could occur.

This stimulation of SCC raised an interesting question: do psychotropic drugs affect both (Na+K)-ATPase and adenylyl cyclase? If so, it is conceivable that an early effect on the cyclic nucleotide system might be subsequently masked by a depression of the (Na+K)-ATPase system. This explanation would fit the biphasic effect on SCC frequently found with high concentrations of harmaline or amitriptyline. It could be argued however that the stimulation and the inhibition of SCC merely reflect parallel phenomena in the Na pump activity, an argument already invoked to account for a biphasic effect of ouabain in toad bladder [33]. To our minds, two facts speak in favor of an increase in cAMP: (i) the nonadditivity of sequential stimulations of SCC by the drug and the hormone; (ii) the vasopressin-like effect of harmaline on water transport, which is discussed next.

Psychotropic Drugs and Water Transport

It is generally admitted that Na and water transport are uncoupled processes in tight epithelia such as toad bladder and frog skin [9]. Permeability to both chemical species is markedly increased by neurohypophyseal hormones which appear to act upon distinct entry pathways at the outer barrier of the epithelia. A common denominator exists, however, prior to this membrane effect: the generation of cAMP by activation of adenylyl cyclase(s).

The hypothesis that the natriferic effect of harmaline is mediated by cAMP received additional support with the finding of a hydrosmotic action of this alkaloid in toad skin [10, 13, 19]. Interestingly, the range of concentrations of harmaline required to stimulate water flow (J_{H_2O}) and SCC was the same (10^{-4}–10^{-3} M). With improved automated techniques [13, 44] it was possible to follow the kinetics of water flow changes with considerable precision, as illustrated in Fig. 3. Harmaline- and vasopressin-induced J_{H_2O} changes were quite similar with respect to time course and maximal J_{H_2O} attained. As previously reported for the stimula-

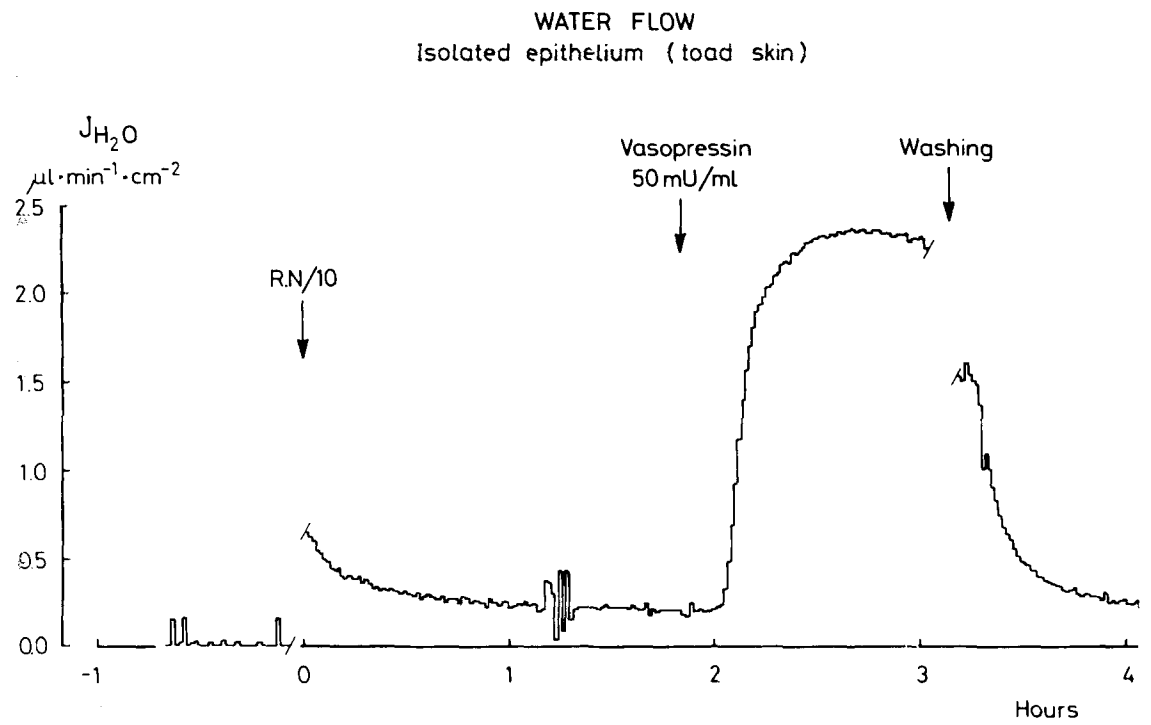

Fig. 3. Measurement of water flow (J_{H_2O}), with an automated method [13], across the isolated epithelium of a toad *Bufo bufo*. The separation from the dermis was obtained by incubating the skin with collagenase. The curve represents the average water flow per minute. *RN*/10 indicates the imposition of an osmotic gradient: internal side—normal Ringer's; external side—same solution diluted 10 times

tion of SCC, the effects of the two agents on J_{H_2O} were not additive. The examples in Fig. 4 are typical although, quite often, 10^{-3} M harmaline induced a maximal increase in J_{H_2O}, that was unaffected by the subsequent addition of vasopressin. The same type of interaction was found between harmaline and norepinephrine, and between harmaline and theophylline. Finally, harmine, an analogue of harmaline, also stimulated J_{H_2O}.

The mechanism of the vasopressin-like effects of harmaline and harmine on water transport is not known. The hypothesis that the hallucinogen increases intracellular cAMP has the appeal of providing for a single explanation of both the natriferic and the hydrosmotic effects. Other mechanisms cannot be ruled out, however, namely, a facilitation of microtubule assembly or a release of lysosomal enzymes, two factors recently implicated in vasopressin-induced water transport [9, 14, 40, 51].

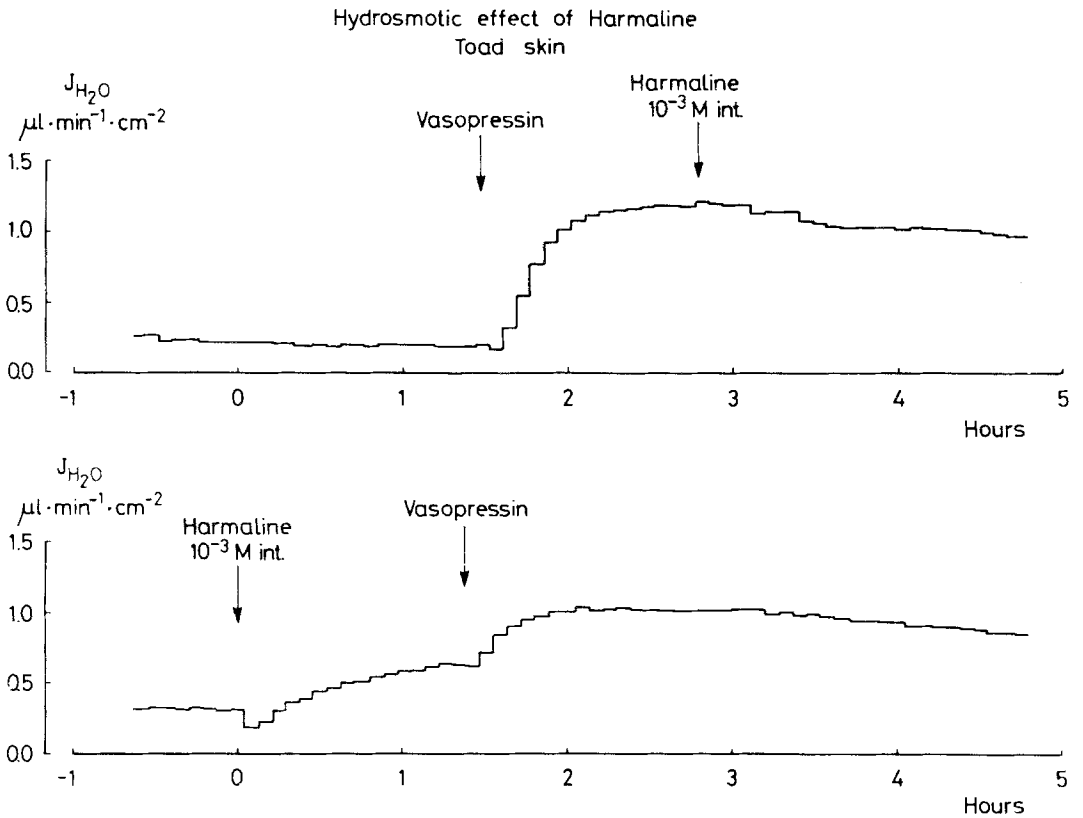

Fig. 4. Vasopressin-like effect of the hallucinogen, harmaline, on water flow. Note the different increments in J_{H_2O}) with and without pre-exposure of the skin to vasopressin (100 mU/ml). The nonadditivity of the hydrosmotic effects of harmaline and vasopressin suggests a common cell target, possibly the cAMP system

If the cAMP hypothesis is retained, an activation of adenylyl cyclase is more likely than an inhibition of phosphodiesterase, the reason being that the hallucinogens increased J_{H_2O} only when present in the internal medium. At this point it is worth mentioning that Berridge and Prince [1] reported a stimulation of fluid secretion in isolated salivary glands of *Calliphora erythrocephala* by another hallucinogen: (+)-lysergic acid diethylamide (LSD). Most importantly, this effect was mediated by cAMP. Attempts were made in our laboratory to measure cAMP in the isolated toad skin epithelia exposed to harmaline. Preliminary experiments, however, revealed an unexpected phenomenon: after treatment with collagenase, the epithelia isolated so far remained sensitive to vasopressin but were insensitive to harmaline (*unpublished observations*). An analogous situation was reported by Crabbé *et al.* [7] for insulin receptors.

The effects of amitriptyline on water transport were rather complex and require further investigation [11]. A transient and weak stimulation of J_{H_2O} was observed sometimes. But the conspicuous feature was an inhibition of vasopressin-induced J_{H_2O}, which was surmountable after 60–90 min of incubation with both agents. Mamelak *et al.* [30] reported that chlorpromazine also inhibits vasopressin- and cAMP-induced water flow in toad bladder. In view of the known interaction of some psychotropic drugs with microtubules [22, 24, 41], it is conceivable that these organelles might be implicated in the effects of amitriptyline and chlorpromazine on water transport.

Another psychotropic agent – lithium – clearly reduces osmotic water flow in kidney and in toad bladder [6]. Similarly, when toad skins were exposed to Li-Ringer on both surfaces, or only to tenfold diluted Li-Ringer's on the external surface, the hydrosmotic response to vasopressin or norepinephrine was abolished or greatly reduced [19]. Interestingly enough, the inhibition of the hydrosmotic effect of harmaline followed the same pattern (Fig. 5). The well-known inhibition of adenylyl cyclase by lithium has been proposed as the mechanism of the hormonal block *in vitro* and of nephrogenic diabetes insipidus *in vivo*. However, a stabilization of microtubules, recently described by Bhattacharyya and Wolff [2], may also play a role and possibly explain the reported block of J_{H_2O} beyond cAMP generation [16].

Psychotropic Drugs and Membrane Calcium

Having observed either inhibitory or stimulatory effects on SCC, according to high or low concentrations of harmaline and amitriptyline added to the internal medium, respectively, we asked ourselves if a similar phenomenon occurred at the outer surface of frog skin. Added to the external medium, both drugs, at high concentrations, depressed SCC, as already shown. In addition, at low concentrations, they did elicit a natriferic effect [11, 13]. An example with amitriptyline is given in Fig. 6. The profile and the magnitude of the stimulation of SCC were variable, but a several-fold rise in current and in electrical potential difference, sustained for more than one hour, was not infrequently seen. Sensitivity of the epithelia varied again considerably, an effect being sometimes observed at 10^{-5} M, but more often between 10^{-4} M and 10^{-3} M.

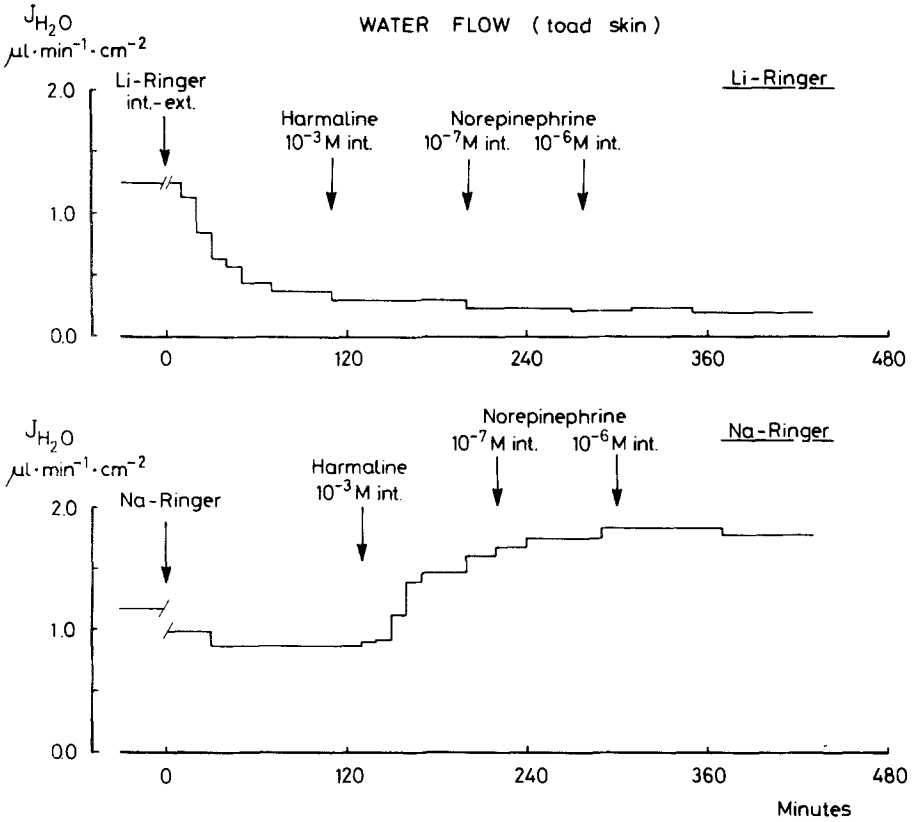

Fig. 5. Effects on J_{H_2O} of the substitution of Li for Na in the Ringer's solution. Note the decrease in basal J_{H_2O} and the inability to stimulate J_{H_2O} with either harmaline or norepinephrine, two hydrosmotic agents in toad skin

This second "vasopressin-like" effect of psychotropic drugs on frog skin had exactly the same features of that reported by this laboratory first with diphenylhydantoin [12] and then with a variety of inorganic cations and organic molecules [9, 31], to which we can add the hallucinogen LSD (*unpublished observations*). The effect could be abolished or prevented by amiloride or ouabain but persisted, although diminished in magnitude, with Li substituted for Na in the outer Ringer's solution.

The interaction of these "external agents" with oxytocin or norepinephrine was particularly interesting. In contradistinction to the results obtained at the internal surface of the skin, exposure to an "external" agent and to one of these hormones in either sequence, led to additive stimulations of SCC [9, 12, 13, 31]. Even if perfect additivity was not

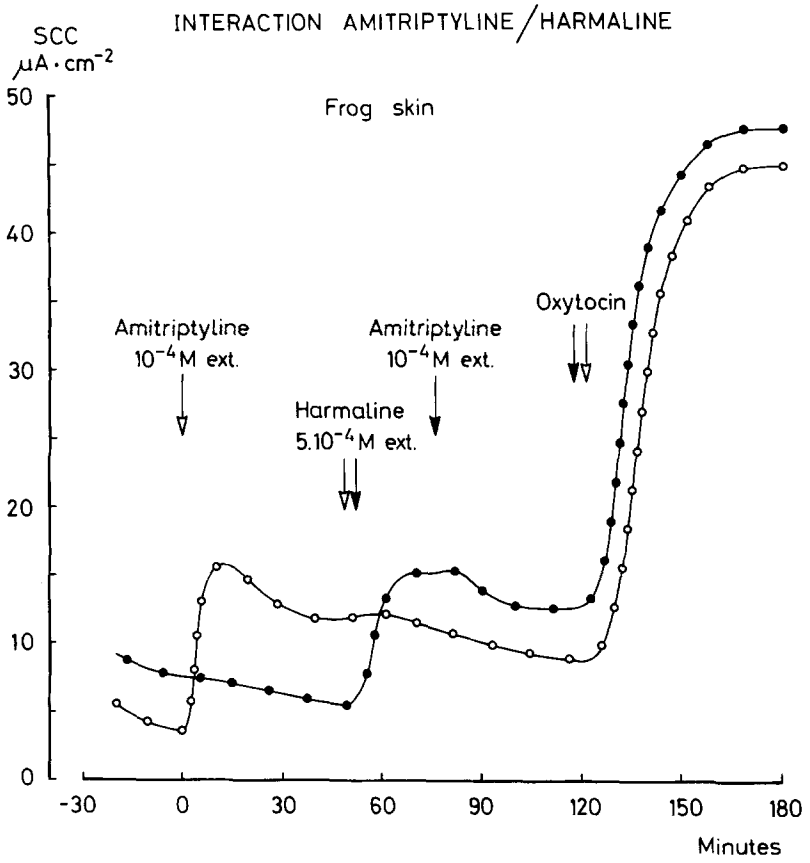

Fig. 6. Stimulation of SCC by amitriptyline or harmaline added to the external solution. Note the mutual inhibition between the two psychoactive drugs contrasting with the normal stimulation of SCC by oxytocin after exposure to both agents

always demonstrated or tested in each instance, a characteristic general feature easily emerged: the possibility of further increasing SCC with an "external" agent in the presence of a maximal, sustained stimulation by oxytocin or norepinephrine [9, 13]. Furthermore, additional work with exogenous cAMP, theophylline and imidazole, suggested very strongly that the effect of "external" agents is independent of cAMP.

Phenomenologically, the natriferic action of "external" agents is the mirror image of the "external Ca effect" described by Curran's group several years ago [23]. Early work with diphenylhydantoin and lanthanides led us to the hypothesis that such agents interact with the "Ca sites" of Curran and, by displacing membrane Ca, open Na entry pathways distinct from those activated by cAMP [9, 12, 31]. At this point,

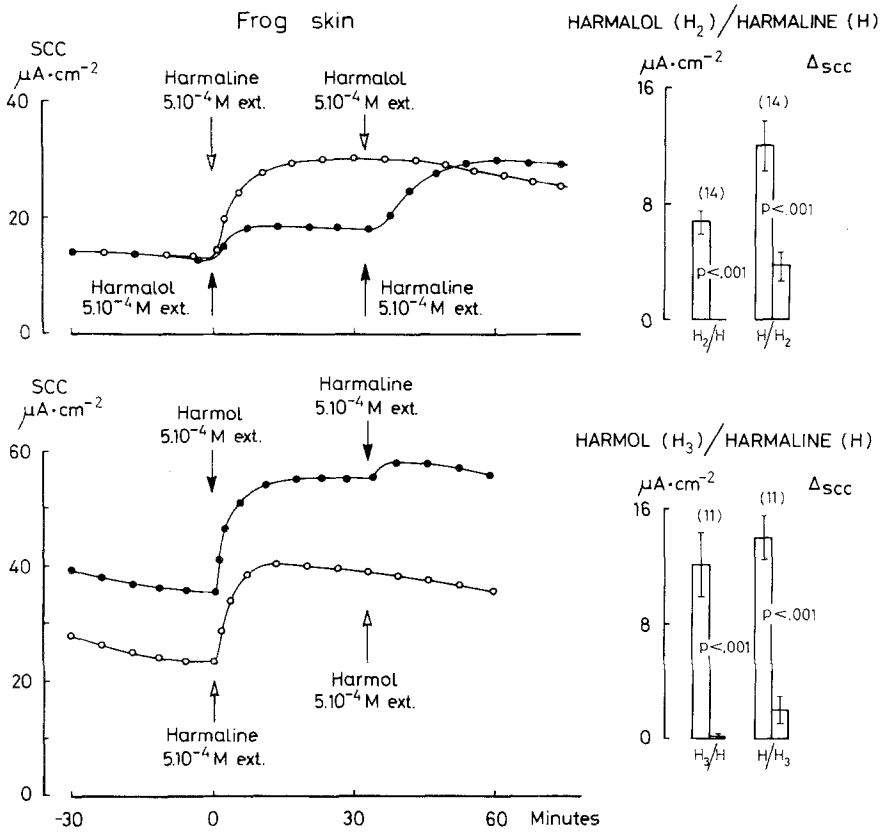

Fig. 7. Mutual inhibition between harmaline and two other harmala alkaloids. The statistical results are summarized at the right side. The notation at the bottom of the bars indicates the natriferic effect of a given agent in the control skin (first bar) and in the skin pre-exposed to the second agent (second bar). Figures in parenthesis = number of paired experiments

it is of interest to note that the ability to interact with membrane Ca^{++} appears to be a property common to all the "external" agents so far studied [9, 13, 18, 31]. Of necessity, the same applies to psychotropic drugs [21, 26, 46].

The question arises as to whether or not the "external" agents have a common receptor site at the outer barrier of the skin. Several lines of evidence speak for a single site. First, there was mutual inhibition not only between harmaline and its analogues (Fig. 7) but also between harmaline and amitriptyline (Fig. 6). Secondly, the same type of interaction was observed between harmaline and other "external" agents, such as propranolol [9, 13, 31], atropine, and ethacrynic acid (*unpublished observations*). Thirdly, the mutual inhibition, shown in Fig. 8, between

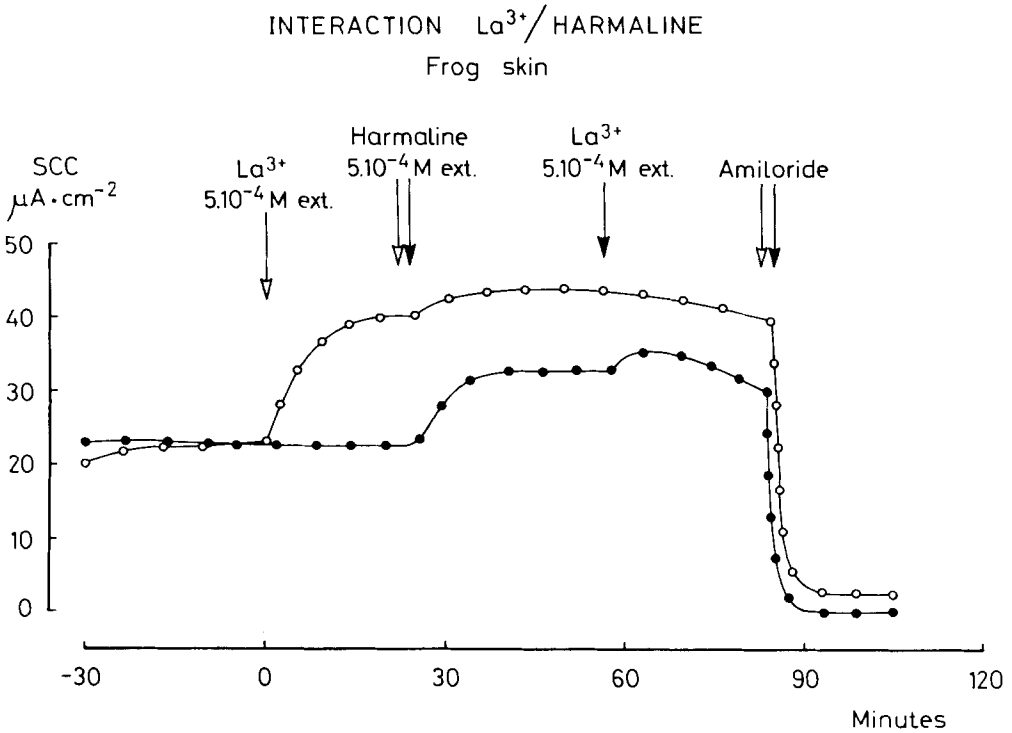

Fig. 8. Mutual inhibition between the natriferic effects of a membrane calcium probe (La^{3+}) and harmaline. The Na-entry sites activated by these "external" agents are sensitive to amiloride. A similar interaction was seen with La^{3+} and amitriptyline

harmaline and lanthanum – a calcium probe [35] – suggests that the receptor site for "external" agents might indeed be a calcium site.

Conclusion

Psychotropic drugs were shown to alter Na and water transport in amphibian membranes. Two main types of effects were found: (i) ouabain-like, on Na transport; (ii) vasopressin-like, on Na and water transport. It is intended that these terms be only descriptive. They imply a phenomenological similitude, not necessarily a mechanistic one. It is likely, however, that psychotropic drugs and the agents they mimic share at least some common targets in the cell machinery. Their exact nature remains to be established.

On Fig. 9 are represented schematically the mode of action of vasopressin, according to current knowledge, and a possible mode of action

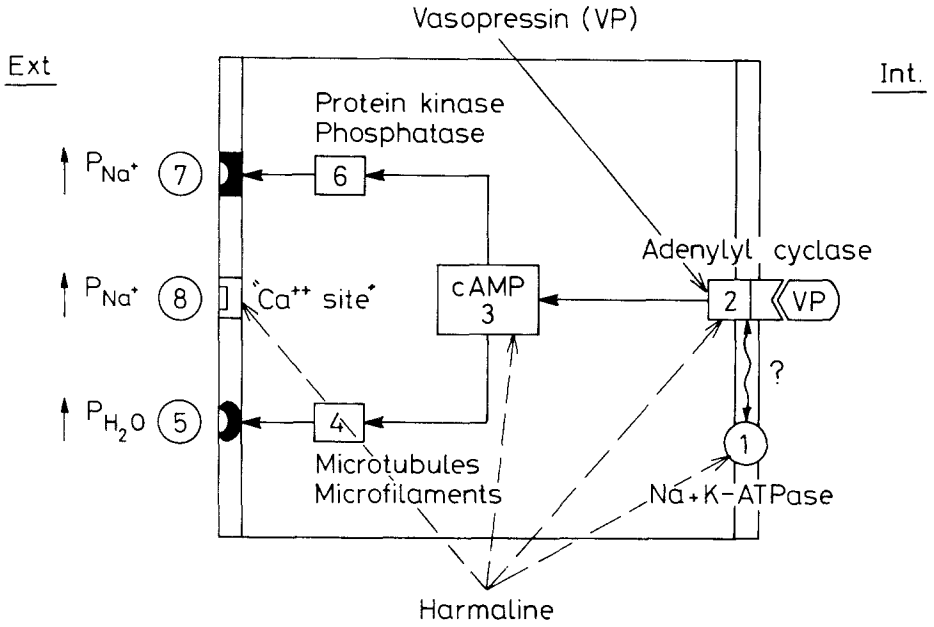

Fig. 9. Schematic representation of an epithelial cell of amphibian skin, sensitive to both vasopressin and harmaline. Four cell targets are considered to explain the multiple effects of harmaline on Na (P_{Na}) and water (P_{H_2O}) permeability (*see* text). A putative increase in cAMP may result from the activation of adenylyl cyclase or from some other nonspecified mechanism. An interaction between adenylyl cyclase and (Na+K)-ATPase is postulated

of harmaline, according to data presented in this paper. One point deserves a final comment: if one accepts that harmaline affects both (Na+K)-ATPase and adenylyl cyclase, is this finding a fortuitous one or does it reveal a normal link between these two key membrane enzyme systems? Work in progress and recent evidence from the literature [17, 28, 36, 45] support the latter hypothesis. Amphibian epithelia may be ideally suited to examine such link.

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