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Vasopressin-Like Effects of a Hallucinogenic Drug – Harmaline – on Sodium and Water Transport

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Summary. To determine if harmala alkaloids affect transport systems other than (Na + K)-ATPase, effects of harmaline on Na and water fluxes were studied in amphibian skins. Net Na flux was evaluated from short-circuit current, and water flux monitored with automatic, volumetric methods. At 2 to 5 mM, harmaline consistently inhibited SCC and prevented the natriferic effects of oxytocin and norepinephrine. However, at 0.1 to 0.5 mM, harmaline produced an increase in SCC inhibitable with amiloride. The stimulatory effects of harmaline and oxytocin were either nonadditive or additive depending on whether the hallucinogen was present in the inner solution or in the outer solution bathing the skin, respectively. Water flow was not modified by harmaline on the outer medium. In contrast, addition of the drug to the inner medium elicited a conspicuous, sustained, vasopressin-like, hydrosmotic effect, comparable to and competitive with those of vasopressin and norepinephrine. The ensemble of these results suggests that harmaline may affect three distinct transport systems: (i) the Na pump; (ii) the cyclic nucleotide system; (iii) the Na entry pathway at the outer membrane of the skin that is also activated by agents such as diphenylhydantoin, lanthanides and propranolol.

It has been reported that the hallucinogenic drug harmaline inhibits Na extrusion from symmetric cells such as nerve and erythrocyte, probably by interacting with the Na sites of (Na + K)-ATPase [2, 3, 12]. In view of the ubiquitous occurrence of this enzyme in both polar and nonpolar cell systems, it appeared justified to investigate the effects of harmaline on Na transporting epithelia. For that purpose, amphibian membranes are generally considered as convenient *in vitro* models for the study of active Na transport in asymmetric cells [7]. In addition, these tissues have also long been used to investigate the stimulus-effect coupling of hormones which modify the permeability of epithelia to a variety of substances [7, 16, 35].

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The results presented in this paper show that, in amphibian skins, harmaline alters the transport mechanisms of at least two chemical species: Na and water. At a high concentration (5 mM) harmaline abolished the transepithelial net movement of Na, an effect compatible with the inhibition of (Na+K)-ATPase previously described in other tissues [2, 3, 12, 21, 26]. Unexpectedly, however, harmaline induced vasopressin-like effects on Na and water transport in skins exposed to lower concentrations of the hallucinogen ($10^{-4}-10^{-3}$ M). The results clearly indicate that harmaline affects cellular transport systems other than the Na pump among which the cAMP system is a likely target. A preliminary report of these data was presented elsewhere [8].

Materials and Methods

Two amphibian species were used—frogs *Rana ridibunda* and toads *Bufo bufo*. As specified in *Results*, most Na transport studies were performed in frog skins while toad skins were mainly used for water flux experiments.

Transepithelial electrical potential difference (PD) and short circuit current (SCC) were monitored continuously by means of standard techniques previously described [10, 18]. The Ussing-type chambers used were double, each compartment being filled with 5 ml of a Ringer's solution of standard composition [1].

The volumetric method to measure water flow J_{H_2O} has also been reported in detail [11, 18, 25]. In most experiments the apparatus used corresponded basically to the original description of the method [25] with improved circuitry. In the remaining experiments, a fully automated technique, recently developed, was utilized, in which a capacitance signal, instead of an optical one, triggered the movement of the carrier placed around the pipette to follow the displacements of the meniscus. The osmotic gradient across the skin was the following: internal side normal Ringer's solution; external side normal Ringer's solution diluted 10 times.

All chemicals used were reagent grade. The commercially available hormones utilized were: Pitressin (Park-Davis) for vasopressin; Syntocinon (Sandoz) for oxytocin; and D−L-norepinephrine (Fluka). Harmaline and other harmala alkaloids (harmine, harmol, harmalol) were purchased from Fluka and from Aldrich-Janssen Pharmaceutica; cyclic AMP from Boehringer; propranolol from Sigma and ICI (Inderal).

All results are given as mean ± SEM. The p values were obtained by means of the Student t-test for paired data.

Results

A) Harmaline (Internal Side) and Sodium Transport

1) High concentrations of harmaline. A marked decrease in SCC and PD was found when the hallucinogen was added to the solution bathing the internal surface of frog skin at concentrations ranging from 1 to

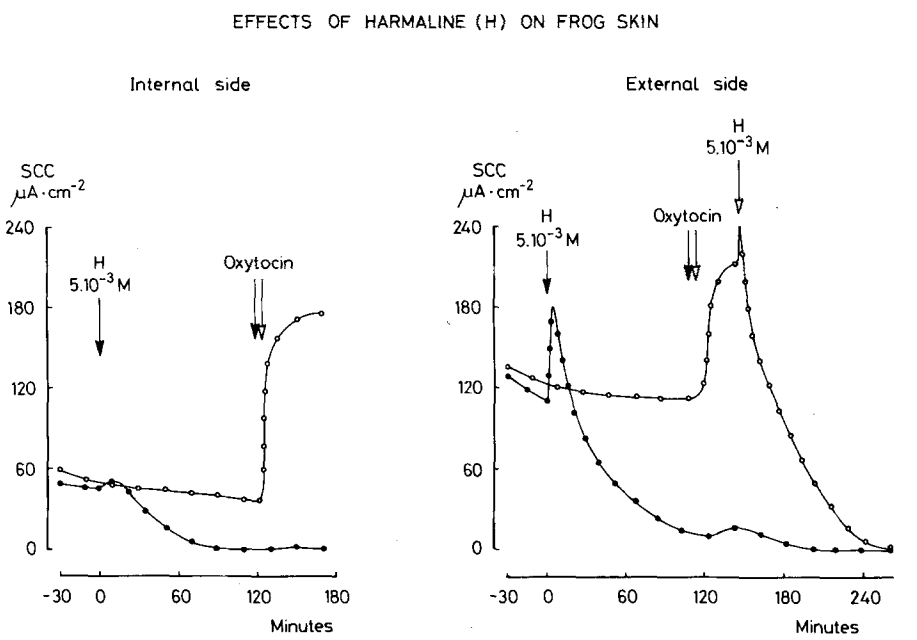

Fig. 1. Inhibition of SCC and block of the natriferic response to oxytocin in frog skins exposed to high doses of harmaline added to the inner or to the outer solution. The drug appeared to be even more effective in the epithelium preexposed to oxytocin. Except for Fig. 4, in this figure and in the following ones, the symbols o—o—o and ●—●—● represent the SCC curves of two paired skins mounted in a double chamber

5 mM. In some experiments, the fall in SCC and PD was preceded by a rise in these two parameters, the magnitude of which varied but could reach 70% of the values recorded before addition of harmaline. Regardless of its magnitude, the rise was never sustained. The subsequent fall of SCC was slow, minimum values being attained after 120–150 min of exposure to harmaline (left side of Fig. 1). The degree of inhibition of SCC was dose-dependent reaching almost 100% at 5 mM harmaline (Fig. 2). Its reversibility was examined by replacing the medium containing harmaline by fresh Ringer's solution (3 washings at intervals of 10 min). Recovery of SCC and PD was very slow and never complete at 5 mM harmaline. At 2 and 1 mM however, despite considerable decreases in SCC averaging 80% and 65%, respectively (Fig. 2), the recovery was much faster with return to the pre-exposure values in most cases.

2) Low concentrations of harmaline. A "vasopressin-like" effect on SCC was observed in frog skins exposed to concentrations of harmaline lower than those reported in the previous section. A typical example is

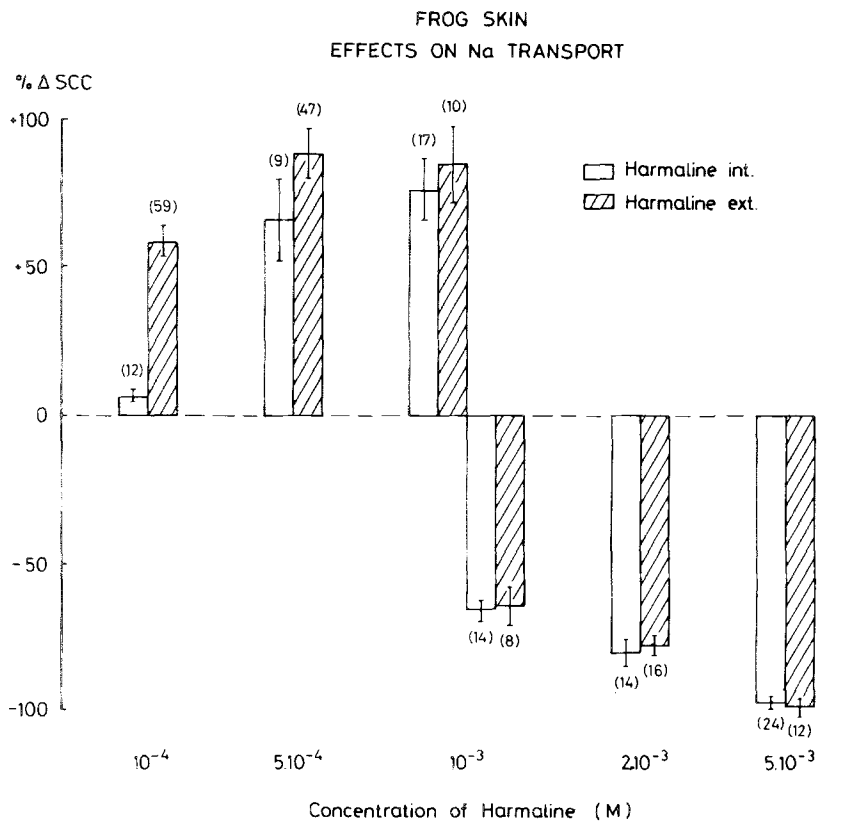

Fig. 2. Stimulatory and inhibitory effects on SCC induced by different concentrations of harmaline added to the inner or to the outer solution. At 10^{-3} M harmaline could elicit either effect according to the sensitivity of the skins. The number of paired studies is indicated in parenthesis. Results (mean ±SEM) are expressed as percentage ΔSCC changes with respect to control values

shown in Fig. 3; the hallucinogen produced a stimulation of SCC quite similar to that induced by oxytocin on a paired piece of the same skin. However, when the concentration of harmaline was raised to 5 mM, SCC fell to zero, as already noted in the previous section.

The natriferic action of harmaline was sustained and dose-dependent (Figs. 2 and 3). A concomitant rise in PD was also seen. Sensitivity of the skins to the drug was quite variable, a detectable stimulation being already noticed at 0.1 mM in some tissues; a maximal stimulation was usually found between 0.5 and 1 mM.

It should be noted, however, that 1 mM harmaline could either stimulate or inhibit SCC (Fig. 2). The ability to inhibit SCC was ap-

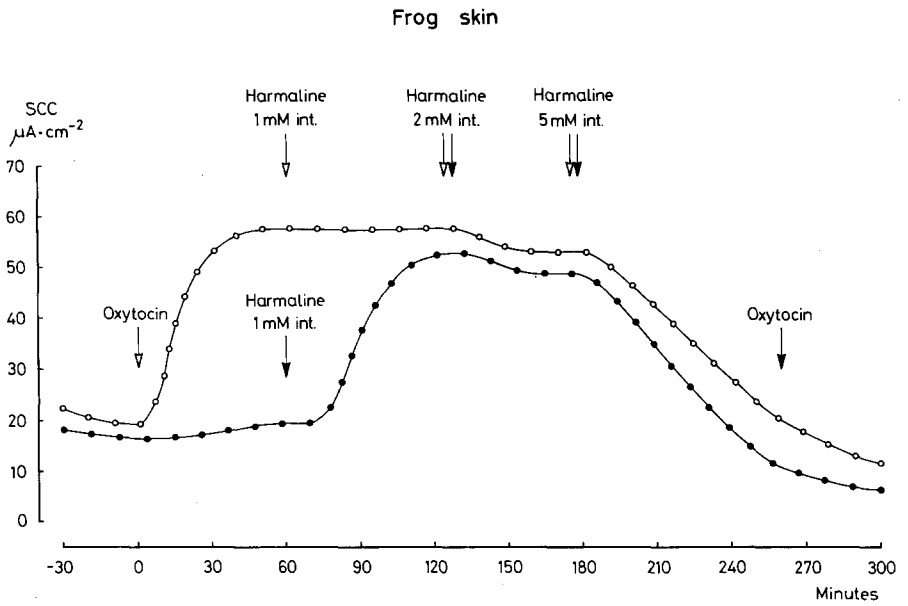

Fig. 3. Vasopressin-like effect of harmaline (1 mM) added to the inner solution. No stimulation of SCC was seen in the piece of skin pre-exposed to oxytocin (50 mU/ml). At 5 mM, the drug inhibits SCC and blocks the response to oxytocin

parently related to the variable degree of permeation of the epithelial cells by harmaline.

3) Interaction of harmaline with hormones. Pre-exposure of frog skins to harmaline added to the internal medium modified considerably the natriferic response to oxytocin. Two types of inhibition could be identified. A first one occurred at concentrations of harmaline depressing SCC per se and resulted in responses to the hormone that were small or nil (Figs. 1 and 3 and Table). Such inhibition, however, was not specific for oxytocin but apparently due to the blockade of the cell Na extrusion mechanism by the alkaloid, as indicated by the fact that similar results were found with norepinephrine, another hormone having a natriferic action. Control ΔSCC values with the catecholamine averaged 13.8 $\pm 3.06\,\mu A \cdot cm^{-2}$, while in tissues pre-exposed to harmaline, no detectable change in SCC was found ($n=7$, $0.01 > P > 0.001$).

The second type of inhibition was observed at concentrations of harmaline stimulating Na transport. As shown in Fig. 3, harmaline (1 mM) and a supramaximal concentration of oxytocin (50 mU/ml) induced similar increments in SCC. Besides, and even more significant is

Table 1. Effect of harmaline on the natriferic action of oxytocin in frog skin

Harmaline	Oxytocin ΔSCC ($\mu A \cdot cm^{-2}$)		n	P
	Control	Experimental		
5×10^{-4} M (int)	48.1 ± 8.38	8.9 ± 3.39	4	$0.02 < P < 0.05$
10^{-3} M (int)[a]	38.0 ± 5.73	12.5 ± 2.15	13	$P < 0.001$
10^{-3} M (int)[b]	30.3 ± 6.60	11.4 ± 4.94	12	$P < 0.001$
5×10^{-3} M (int)	41.4 ± 4.53	0.5 ± 0.36	19	$P < 0.001$
10^{-4} M (ext)	37.5 ± 3.88	35.6 ± 5.19	14	$P > 0.6$
5×10^{-3} M (ext)	25.9 ± 5.92	0.4 ± 0.32	8	$0.01 < P < 0.001$

Increments in SCC (ΔSCC) induced by oxytocin in control skins and in paired experimental skins pre-exposed to harmaline added to the internal (int) or to the external (ext) bathing solution. At 10^{-3} M, harmaline had a stimulatory effect on SCC in one group ([a]) and an inhibitory effect in the other ([b]). n = number of paired skins; P values from Student t-test.

the fact that, after pre-exposure of the skin to oxytocin, harmaline did not increase SCC any further. The interaction between the natriferic effects of oxytocin and harmaline was examined at different concentrations of the hallucinogen. At 10^{-4} M, harmaline had no observable effect per se on SCC and did not interfere with the stimulation induced by oxytocin. However, at higher concentrations of harmaline ($5 \times 10^{-4} - 10^{-3}$ M), the oxytocin-induced rise in SCC was diminished with respect to control as summarized in the Table.

In view of the fact that toads *Bufo bufo* were routinely used for water flow studies, a series of SCC experiments was performed with the skin of this amphibian species. Again, harmaline induced a natriferic action nonadditive to that of oxytocin.

B) Harmaline (External Side) and Sodium Transport

1) High and low concentrations of harmaline. Experiments were carried out to examine the effects of the presence of harmaline in the solution bathing the outer surface of frog skin. Results showed striking similarities with those previously obtained with harmaline in the inner solution, although the mechanisms involved appeared to be different in the case of the natriferic response.

Addition of 5 mM harmaline induced a marked fall in PD and SCC which was frequently preceded by a transient rise of both parameters,

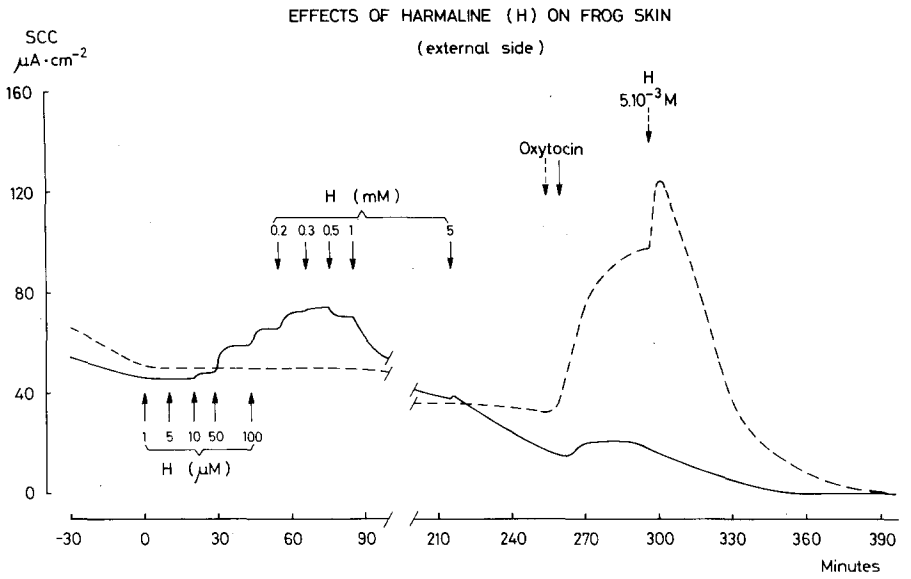

Fig. 4. Dose-response curve to harmaline added to the outer solution bathing frog skin. Depending on the dose, SCC could be either stimulated or inhibited. The time-course of the inhibition is faster after pre-exposure to oxytocin (*see also* Fig. 1). The continuous and the interrupted lines represent the SCC curves of two paired skins

quite conspicuous some times but always of short duration (right side of Fig. 1). Minimum values of SCC were attained after 120–150 min of exposure to harmaline. Fig. 2 shows the average decrements of SCC at different concentrations of harmaline. Study of the reversibility of this inhibition revealed a pattern similar to that already described for the inner side.

At lower concentrations the hallucinogen stimulated SCC. Some skins already reacted to 5×10^{-6} M of harmaline. The rise in PD and SCC was rapid, sustained, and dose-dependent (Fig. 4). The percentage increases in SCC for the concentrations of harmaline most frequently used are indicated in Fig. 2. Depending on the responsiveness of the epithelia, harmaline at 5×10^{-4} or 10^{-3} M provoked stimulation, inhibition, or a biphasic change of PD and SCC.

The natriferic effect of harmaline was easily reversible by simple washing with fresh Ringer's solution; iterative stimulation of SCC could be induced without apparent modification of the pattern of the response (Fig. 5). Previous exposure of the skin to amiloride (10^{-5} M) prevented the natriferic action of harmaline, which was readily re-established by removal of the diuretic from the outer medium. Both findings suggest that

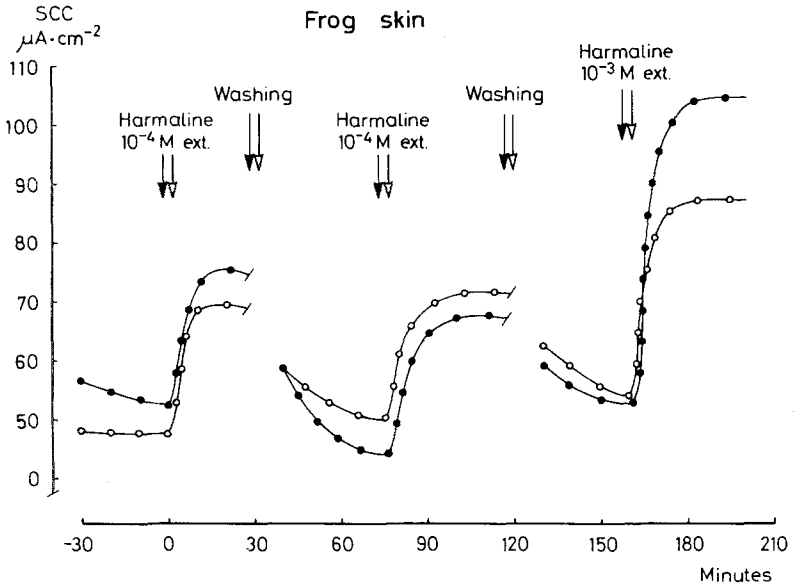

Fig. 5. Iterative stimulation of SCC by harmaline. Return to control values was achieved by replacing the external solution containing the drug with fresh Ringer's solution

harmaline-induced stimulation of SCC was accounted for by a commensurate rise in net Na flux.

2) Interaction of harmaline with hormones. The nature of the interaction between the effects of harmaline and oxytocin on SCC depended on the concentration of harmaline in the external medium, as shown in the Table and in Figs. 4 and 6.

At high concentrations of the hallucinogen (5×10^{-3} M) there was a marked inhibition of the hormonal effect of the order of 98 % (Table and Fig. 4). At low concentration (10^{-4} M), harmaline had a natriferic effect per se but did not modify statistically the subsequent stimulation of SCC by oxytocin (Table and Fig. 6). Conversely, the action of harmaline (Fig. 6) was not significantly modified by previous exposure of the skin to supramaximal concentrations of the hormone. Similar conclusions were drawn from experiments performed with harmaline and norepinephrine or a combination of norepinephrine and oxytocin.

In view of the fact that the natriferic action of these two hormones is mediated by cAMP, it appeared justified to examine the effects of harmaline with and without pre-exposure of the skin to the nucleotide. Again, no statistical difference was found between control and experi-

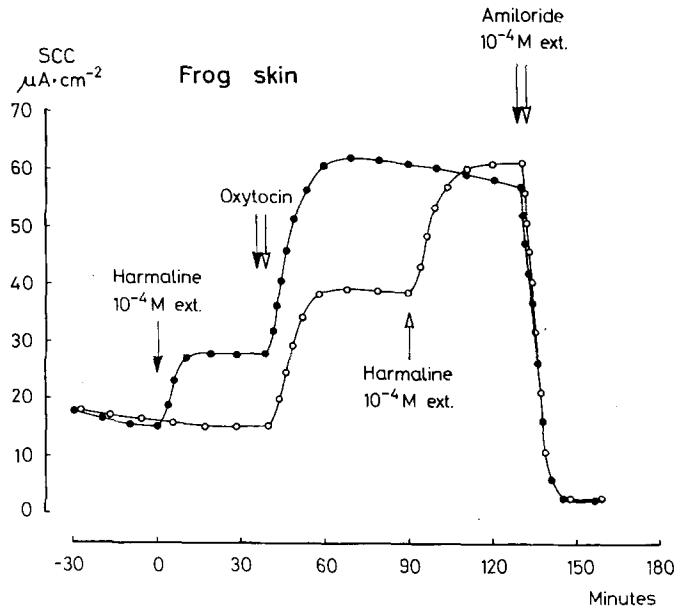

Fig. 6. Additive stimulations of SCC induced by harmaline (external side) and oxytocin presented to frog skin in two different sequences. Note that harmaline increases SCC after maximal stimulation with oxytocin (50 mU/ml). SCC remains totally inhibitable by amiloride

mental tissues, which reinforces the hypothesis that the stimulation of SCC by harmaline added to the outer solution is independent from the cAMP system.

3) Interaction of harmaline with propranolol. Both the pattern of the response to harmaline and its apparent independence from cAMP resemble very much the effects previously obtained in this laboratory with agents such as diphenylhydantoin, lanthanides, and propranolol [7, 10, 18]. To investigate this point further, a series of experiments was designed to examine the interaction between the effects of propranolol and harmaline, both added to the external medium. Results are summarized in Fig. 7 and suggest competition of the two substances for similar Na entry sites at the external surface of frog skin.

4) Analogues of harmaline. Several analogues of harmaline were tested – harmine, harmalol, harmol. Their stimulatory effects on frog skin were qualitatively very similar to those reported here for harmaline, but for some differences in potency. In general, harmine was more potent

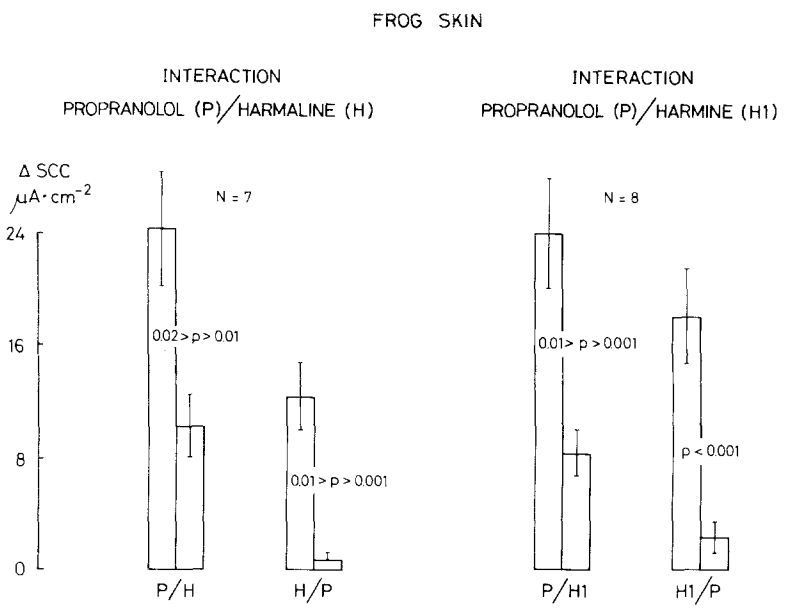

Fig. 7. On the left side of the figure, the notation *P/H* indicates increments in SCC produced by propranolol in the absence and in the presence of harmaline; conversely *H/P* indicates increments in SCC produced by harmaline in the absence and in the presence of propranolol. *Mutatis mutandis* for propranolol and harmine, on the right side of the figure

than harmaline (*see* Fig. 7, for example), while harmalol and harmol were less potent. Their interaction either with oxytocin or with propranolol yielded results compatible with the hypothesis that harmaline and the other harmala alkaloids so far tested induce effects which are additive to those of oxytocin, but competitive with those of so-called "Ca-displacing agents" (Fig. 7).

C) Harmaline and Water Transport

Addition of harmaline to the internal medium bathing the ventral skin of toads *Bufo bufo* induced a conspicuous, "vasopressin-like" effect on water transport (Fig. 8). Stimulation of the hydrosmotic flow was already detectable at a concentration as low as 10^{-4} M and increased in a dose-related manner, a maximal response usually being obtained at concentrations of harmaline lying between 5×10^{-4} and 10^{-3} M. This effect was highly significant. At 5×10^{-4} M, harmaline increased J_{H_2O} (in $\mu l \times min^{-1}$) from 1.14 ± 0.14 to 2.59 ± 0.22 ($n = 22$, $P < 0.001$). Likewise, at 10^{-3} M, J_{H_2O} changed from 1.20 ± 0.08 to 2.74 ± 0.18 ($n = 76$, $P < 0.001$).

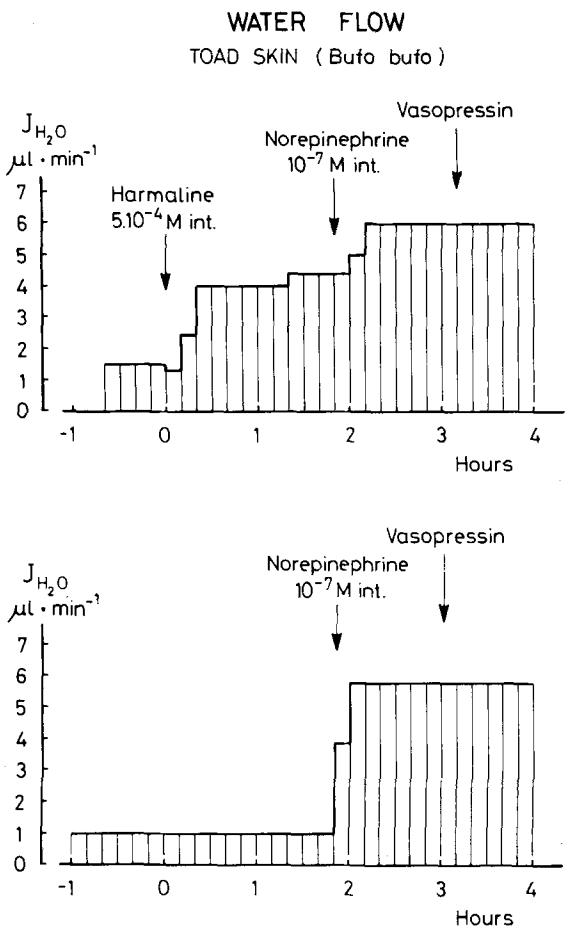

Fig. 8. Vasopressin-like, hydrosmotic effect of harmaline added to the inner solution bathing toad skin. Note that the additional increase in J_{H_2O} elicited by norepinephrine and vasopressin is small when compared to the effect in the control, paired tissue

The stimulation of water flow was sustained, but its kinetics showed some variation. Most skins responded within 5 to 10 min upon exposure to harmaline and reached a plateau in 15–20 min, while others exhibited a sluggish reaction with a smooth and progressive increase in water flow during 1 to 2 hr. No obvious reason was found to explain these two types of response.

Results qualitatively similar to those obtained with *Bufo bufo* were also seen in a few experiments performed with the skin of *Rana ridibunda*. However, as it is well known, hydrosmotic effects are highly variable and difficult to reproduce in frog skin, and, consequently, they were not investigated in more detail with harmaline in this epithelium.

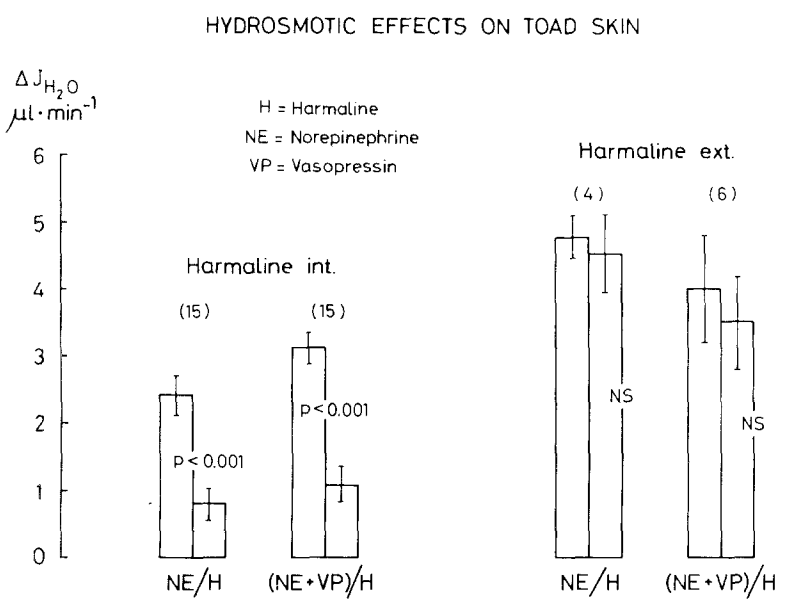

Fig. 9. Comparison of the hydrosmotic action of NE (or NE+VP) in control epithelia (first bar) and in epithelia pre-exposed to harmaline (10^{-3} M) added to the internal or to the external solutions (second bar). Number of paired studies is indicated in parenthesis

In view of the "vasopressin-like" actions of harmaline on both Na and water transport, we studied next the interaction of its hydrosmotic effect with that of hormones such as vasopressin and norepinephrine. Fig. 8 shows the sustained hydrosmotic effect induced by 5×10^{-4} M harmaline, which accounted for 65% of the total response of a tissue challenged sequentially with harmaline, norepinephrine (10^{-7} M), and a supramaximal concentration of vasopressin (100 mU/ml). Very often, 10^{-3} M harmaline stimulated water flow in such a manner that the subsequent addition of norepinephrine and vasopressin was practically without effect. These findings, taken together with the results summarized in the left part of Fig. 9, suggest that there is competition between the hydrosmotic action of harmaline and that of both hormones. Further evidence in favor of such hypothesis was provided by the observation (not shown) that harmaline had no effect on water flow when added to a tissue maximally stimulated by vasopressin or norepinephrine.

In contrast, harmaline added to the outer solution had no effect on water permeability. This applied not only to basal hydrosmotic flow, but also to the hormone-induced water flow, as shown on the right part of Fig. 9.

Discussion

The results presented in this paper show for the first time that the hallucinogenic drug, harmaline, has the property of modifying the permeability of polar epithelia not only to Na but also to water.

In what concerns Na, two opposite effects of harmaline were found: inhibitory and stimulatory. Both could be elicited whether the drug was added to the inner or the outer Ringer's solution. The evidence obtained here suggests that the blockade of Na movement can be explained by a single mechanism, very likely the inhibition of the (Na+K)-ATPase of the epithelial cells. In contrast, two distinct "vasopressin-like" effects were disclosed in the stimulatory action of harmaline: (i) a first one, elicited by harmaline added to the inner solution, was nonadditive to the hormone-induced stimulation of Na transport and possibly mediated by cAMP; (ii) a second one, elicited by harmaline present in the external solution, was additive to the hormonal natriferic effect and apparently independent of the cAMP system.

The inhibition of Na transport, as judged from the decline in SCC, was slow and required high concentrations of harmaline (5×10^{-3} M) in the internal or in the external media to attain a maximal effect (Figs. 1 and 4). These observations agree with results described by others in amphibian epithelia [3, 13, 14, 21] and in other tissues [2, 3, 12, 26] and have been generally interpreted as a consequence of the inhibition of the ATPase involved in the cell Na extrusion mechanism. The need for high concentrations of harmaline to provoke a maximal inhibition of SCC is probably related to a rather difficult permeation of both the inner and the outer membranes of the epithelial cells by the drug. Consequently, high concentration gradients of harmaline must be imposed across the plasma membrane to achieve effective intracellular concentrations, a phenomenon particularly well studied in red blood cell [12]. It also implies an intracellular site of action to induce the inhibitory effect of harmaline on the Na pump, an interpretation consonant with current views on the sidedness of the plasma membrane and of the (Na+K)-ATPase embedded in its lipid matrix [27, 34]. As shown in Fig. 1, the kinetics of SCC inhibition is faster in skins pre-exposed to oxytocin, suggesting a facilitation of the entry of harmaline through the external membrane in this experimental setting. Similar results were found in this laboratory with amitriptyline [7], an effect reminiscent of the general increase in permeability of the luminal membrane to a variety of drugs in toad bladders stimulated by vasopressin [17].

Earlier reports suggested a direct interaction between harmala alkaloids and the Na site not only of the (Na+K)-ATPase [3], but also of the membrane component ("carrier") involved in the cotransport of Na and glucose or amino acids in intestine and kidney proximal tubule [29–33]. More recent work has challenged this interpretation [4, 21, 24, 26] and showed in addition an effect of harmaline on other ATPases [26]. Our results are compatible with the alleged inhibition of the (Na+K)-ATPase from the cytoplasmic side of the plasma membrane, but do not provide a clue for a specific interaction between harmaline and Na.

The amiloride-sensitive natriferic effect of harmaline added to the inner solution very much resembled the stimulation of SCC produced by oxytocin in amphibian skins. The onset of the response, its time-course, and the percentage increase in SCC were often similar with both agents either in frog skin (Fig. 3) or in toad skin. Besides, harmaline- and oxytocin-induced stimulations were nonadditive, as shown in the Table and in Fig. 3. Such interaction is to be distinguished from that observed at concentrations of the hallucinogen which inhibit the Na pump (Figs. 1 and 3 and Table). In the latter case, Na movement across the epithelium is diminished by the partial or total block of the extrusion mechanism, regardless of the processes that might have activated Na entry in the epithelial cells or any other steps acting prior to the Na pump.

Both the characteristics of this natriferic effect of harmaline and its interaction with the natriferic effect of oxytocin are compatible with an interaction between harmaline and the cAMP system, without excluding, however, an interplay with other cell systems.

The second type of stimulatory effect of harmaline on SCC described in this paper had a feature in common with the one just discussed: amiloride-sensitivity. In many other respects, however, the two effects differed considerably, namely, kinetics, interaction with oxytocin, and surface of the epithelial cell primarily affected by the drug.

Comparison of the "internal" and "external" natriferic effects of harmaline shows that the latter had a much faster onset, a lower threshold (less than 5×10^{-6} M in some skins) and, for concentrations in the range of 10^{-4} M, the percentage increase in SCC was considerably higher (Fig. 2). A most distinctive feature, however, was the interaction with oxytocin. In contrast with the nonadditivity of the effects reported on Fig. 3 and in the Table when the hallucinogen was present in the *inner* medium, harmaline in the *outer* medium induced an additive, sustained rise in SCC in epithelia maximally stimulated by oxytocin (Fig. 6 and Table). Such findings prompted the systematic comparison of the "exter-

nal" natriferic effects of harmaline in resting skins and in skins activated by either endogenous cAMP (following exposure to oxytocin or norepinephrine) or by exogenous cAMP itself, added to the internal medium. In each instance, there was no significant difference between the average ΔSCC values in the two conditions. Likewise, as shown in the Table, no difference was found in the stimulation of SCC induced by oxytocin in control skins and in skins pre-exposed to harmaline.

The "external" natriferic action of harmaline, including its additive interaction with neurohypophyseal hormones, can be viewed as the mirror image of the effects of calcium on the outer surface of frog skin, described by Curran *et al.* [5, 6, 15] some years ago. Moreover, as already pointed out in *Results*, the effect of harmaline (and other harmala alkaloids) is indistinguishable from that previously found in this laboratory with a series of agents capable of increasing the permeability of the outer membrane of frog skin, namely diphenylhydantoin, lanthanides and propranolol [7, 10, 18]. Consistent with such similarity, and also indicating a common site of action, is the mutual inhibition observed between harmala alkaloids and propranolol when these drugs were added sequentially to the outer medium (Fig. 7). Finally, it should be noted that the Na nature of the effect on SCC of several "external" agents, including harmaline, has been directly proved by the finding that they induce equivalent increments in SCC and in net Na flux in frog skin [14, 20, 36].

Circumstantial evidence suggests that the "calcium sites" described by Curran *et al.* [5, 6, 16] may correspond to the Na entry sites activated by drugs exerting a natriferic action from the outer medium [7, 10], all of which have been shown to affect membrane calcium fluxes [7, 18, 19, 22, 23, 28, 37]. In any event and regardless of their putative interplay with calcium, harmaline and similar "external" natriferic agents promote Na entry in the epithelial cells by interacting with membrane sites which are amiloride-sensitive but appear to be distinct from the sites activated by cAMP [7]. Available evidence also indicates that the "external" natriferic action of harmaline in frog skin bears no obvious relationship with the reported effects of the hallucinogen on the luminal membrane of intestine and kidney proximal tubule [29–33].

At first sight, the sustained, potent, "vasopressin-like", hydrosmotic action of "internal" harmaline is surprising, coming from a drug previously considered as having a competitive interaction with Na [2, 3, 12, 31]. It seems to us, however, that this action on the permeability of the skin to another chemical species – water – is intimately related to one of

the effects of harmaline on Na transport, specifically to the "internal" natriferic effect. In both instances, the effects of the hallucinogen not only mimicked those of oxytocin and norepinephrine but also interfered with them (Table and Fig. 3 for Na transport, Figs. 8 and 9 for water transport). Phenomenologically, these two effects are vasopressin-like, but their mechanism(s) of action may differ, of course, from that of the hormone. An interaction between harmaline and the cAMP system appears to be, however, the simplest hypothesis accounting for the increase in both Na and water transport.

In summary, the ensemble of our results indicates three possible points of impact of harmaline in amphibian epithelia: (i) the (Na+K)-ATPase, in connection with the inhibition of Na transport; (ii) the permeability to Na of the outer membrane, in connection with the "external" natriferic effect; (iii) the cyclic nucleotide system of Sutherland, in connection with the hormone like stimulation of Na and water transport from the inner side. Many other substances, including psychotropic drugs such as amitryptiline, affect the first two systems [7, 9]. The third type of action, however, appears hitherto unreported. It deserves further study, including, obviously, the direct measurement of the cyclic nucleotides in isolated epithelia exposed to harmaline.

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