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STRUCTURE-ACTIVITY RELATIONSHIPS OF INTRACEREBRALLY INJECTED TREMORIGENIC INDOLE ALKALOIDS

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Summary—The indole alkaloids harmane, harmine, ibogaine, iboxygaine and ibogaline caused tremor in mice when injected intracerebrally. Harmol, voacangine, voacristine and conopharyngine were inactive in this respect.

Chemical structure strongly influences tremorigenic potency which is increased by a methoxy group and reduced or abolished by a hydroxy or carbomethoxy group.

The great importance of chemical structure for tremorigenic power of indole alkaloids points to specific receptors for these drugs in the brain.

Recently we have shown that the tremor-producing power of some harmala and iboga alkaloids is much more influenced by chemical structure than by lipid solubility, which points to specific receptors for indole compounds in tremorigenic brain structures (ZETLER, SINGBARTL and SCHLOSSER, 1972; SINGBARTL, ZETLER and SCHLOSSER, 1972). Tremorigenic activity was considerably enhanced by the introduction of a methoxy group into the indole nucleus. In the same study, harmalol caused tremor in mice only after intracerebral but not after intravenous application. The reason for this discrepancy is the very low lipid solubility which prevented this compound, after i.v. injection, from reaching brain concentrations sufficiently high to elicit tremor.

The example of harmalol clearly demonstrates that intracerebral injection is a means of overcoming important pharmacokinetic obstacles which perhaps prevented the indole alkaloids voacangine, voacristine, and conopharyngine from causing tremor in mice after subcutaneous injection (ZETLER, 1964). This inactivity is in strong contrast to the tremorigenic activity of the closely related alkaloids ibogaine, iboxygaine, and ibogaline. It was therefore necessary to elucidate, with the aid of intracerebral application, whether (a) the alkaloids just mentioned are in fact devoid of tremorigenic power, and (b) the importance of the methoxy group emerges from this type of experiment, too. These points are obviously relevant to the hypothesis that there exist central receptors for the indole structures in question.

METHODS

Animals

Male albino mice, 22–27 g in weight and of the NMRI-strain, were bought from R. Bäumler (Wolfratshausen) and kept in groups of 25 at an ambient temperature of 23°C. The experiments were performed in the same room at the same temperature. Each animal was used only once and had free access to food (Altromin pellets) and tap water.

Experiments

A micro-syringe (Glenco Scientific Inc., Houston, Texas; catalogue-Nr. 19913–10) was used for intracerebral injections. The cannula (catalogue-Nr. 19920–126; outer diameter 0·6 mm) was equipped with a piece of cork leaving 3 mm of it free for insertion. The site of perpendicular injection was 2–3 mm ventrolateral from the point of intersection of the sagittal suture and a line drawn through the anterior base of the ears. The injection volume was 10 μl. Low solubility of some alkaloids, and the necessity to administer large amounts of inactive compounds to ascertain inactivity, did not allow the injected volume to be reduced. Experiments using ink showed that the point of injection was located in the caudal third of brain, and that the injected fluid spread through both lateral ventricles staining the basal ganglia of both sides and in a few cases the cerebellum.

After injection each mouse was seated in a glass cylinder on a petri dish covered with filter paper, and watched by two observers. Groups of 5 animals were injected and observed for at least 15 min in case of drugs with known tremorigenic action, but for 60 min in case of inactive drugs or control injection of saline. Ten animals were used for each dose and 3–6 doses were given for each drug. Quantal responses were obtained, animals being considered reactors even if tremor was present only for seconds.

Substances and solutions

Source and molecular weight (mol. wt.) of a drug are indicated in parentheses. Harmane hydrochloride (mol. wt. 219; C. Roth, Karlsruhe, Germany), harmine hydrochloride (mol. wt. 285; Fluka, Buchs, Switzerland), harmol hydrochloride (mol. wt. 235; Fluka), ibogaine hydrochloride (mol. wt. 347; Aldrich, Milwaukee, U.S.A.), ibogaline (mol. wt. 341; Geigy, Basel, Switzerland), iboxygaine (mol. wt. 326; Geigy), conopharyngine (mol. wt. 399; Geigy), voacangine (mol. wt. 369; Geigy), voacristine (mol. wt. 385; Geigy). Doses refer to the bases. The bases could be solved and kept in solution only if the 0·9% saline was acidified to pH 1·4 with the aid of 1 N sulphuric acid. Therefore, all drug solutions were brought to this pH, and control injections were given of normal and acidified saline.

Statistics

Doses producing tremor in 50% of the animals (ED_{50}) were calculated using the method of LITCHFIELD and WILCOXON (1949). Differences beween means were considered statistically significant if P was $<0{\cdot}05$.

RESULTS

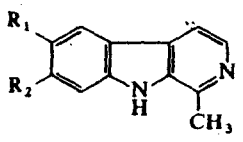
Control injections

In the control experiments with normal saline the behavior of the animals was inconspicuous during the 60 min post injection. Acidified saline (pH 1·4) produced jumping fits in 3 animals ($n = 10$) and a lethal tonic convulsion in 1 animal. However, tremor did not occur during 60 min.

Drugs with tremorigenic activity

Harmane, harmine, ibogaine, ibogaline, and iboxygaine caused tremor, harmine and ibogaline being the most active alkaloids (see Table 1). These actions were dose-dependent and gave parallel dose–response lines with all substances except iboxygaine whose line was steeper. There were at least 80% reactors for the highest dose of each compound. Tremor

Table 1. Chemical structure of indole alkaloids, values of tremorigenic ED_{50} after intracerebral injection, and latency period of tremor



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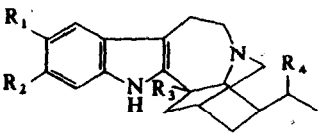

II

	Alkaloid	R_1	R_2	R_3	R_4	Intracerebral ED_{50} μg/animal	Intracerebral ED_{50} nmol/g brain*	Latency period† min
	Harmane	H	H			47·3 34·6–64·7‡	541	1·1 0.9–1·2‡
I	Harmine	H	OCH_3			7·4§ 5·2–10·4	65	1·1 1·0–1·2
	Harmol	H	OH			||		
	Ibogaine	OCH_3	H	H	H	17·6 12·7–24·4	127	1·1 0·9–1·2
	Iboxygaine	OCH_3	H	H	OH	69·2§ 58·1–82·5	532	3·2§ 2·7–3·6
II	Ibogaline	OCH_3	OCH_3	H	H	5·2§ 3·6–7·3	38	4·3§ 3·4–5·1
	Voacangine	OCH_3	H	$COOCH_3$	H	||		
	Voacristine	OCH_3	H	$COOCH_3$	OH	||		
	Conopharyngine	OCH_3	OCH_3	$COOCH_3$	H	||		

* Calculated taking $399 \pm 2{\cdot}5(\bar{x} \pm s_{\bar{x}}; n = 45)$ mg as mean weight of a fresh mouse brain.
† Time from injection till occurrence of tremor.
‡ Fiducial limits for $P = 0{\cdot}05$.
§ Statistically significant ($P < 0{\cdot}05$) difference from harmane or ibogaine, respectively.
|| No tremor observed after doses up to 200 μg/animal.

began within the first 5 min post injection with longest latency periods for iboxygaine and ibogaline. Latency periods were not dependent on dose, which permitted the calculation of means from all animals showing tremor with one given drug. Tremor was interrupted by quiet periods and occurred in many animals, especially after low doses, as a single attack lasting less than 1 min.

All drugs caused the Straub tail phenomenon in many animals. The highest dose of ibogaline (10 μg) led to lethal clonic convulsions in 5 out of 10 mice. Tremor occurred in four of these animals 5–10 min prior to convulsion, total tremor frequency after this ibogaline dose being 80%. 3 and 5 μg of ibogaline elicited vertical jumping in many animals.

Drugs not causing tremor

No tremor was observed after doses of up to 200 μg of harmol, voacangine, voacristine and conopharyngine. However, these drugs were by no means completely without central activity. They produced in many mice the Straub tail phenomenon and jumping fits. Harmol and voacristine caused, after 200 μg, lethal tonic convulsions (harmol: 30%; voacristine: 50%). Conopharyngine and voacristine produced head shaking in some mice during the

first minutes after injection and, 10–15 min later, slowed the respiration with animals assuming a hunched-up position. Catalepsy occurred in some animals after voacangine, voacristine and conopharyngine. There was salivation and diarrhoea after voacangine and conopharyngine. Harmol elicited attacks of stereotyped scratching and grooming of the head region, which lasted about 60 min but were most frequent and strongest during the first 30 min.

DISCUSSION

The efficacy of the intracerebral injection can be appraised by comparing the corresponding brain concentrations with those at the end of tremor caused by peripheral application (Table 2). Ranking orders are identical for both types of brain concentrations: ibogaline

Table 2. Parameters of tremorigenic actions, as mentioned in the discussion

	Concentration in brain			Latency period (min)		Distribution coefficient*
	Intracerebral injection (I.i.)*	Peripheral injection (P.i.)†		Time from injection till occurrence of tremor after:		
	ED_{50}	Concentration in brain at end of tremor				*n*-Heptane/ phosphate buffer (1/15 M; pH 7·4)
	nmol/g	nmol/g	I.i./P.i.	intracerebral injection	10 mg/kg† i.v.	
Harmane	541	243	2·2	1·1	‡	0·475
Harmine	65	25	2·6	1·1	0·38	0·305
Ibogaine	127	63	2·0	1·1	0·34	27·976
Iboxygaine	532	129	4·1	3·2	0·70	0·378
Ibogaline	38	4	9·5	4·3	0·98	0·866

*Taken from Table 1.
†Taken from ZETLER *et al.* (1972; fluorimetric determination).
‡Dose too low to produce tremor.

< harmine < ibogaine < iboxygaine < harmane. Thus, intracerebral injection proved to be an appropriate method for the assessment of structure-activity relationships of tremorigenic indole alkaloids

Our present results confirm the previous reports according to which voacangine, voacristine and conopharyngine, when given to mice peripherally, are devoid of tremorigenic, although not of other central, potency (ZETLER and UNNA, 1959; VOGEL and UEBEL, 1961; ZETLER, 1964; CARROLL and STARMER, 1967). This applies also to harmol in rats (GUNN, 1935; FUENTES and LONGO, 1972). Harmane causes tremor in mice (SIGG, GYERMEK, HILL and YEN, 1964; ZETLER *et al.*, 1972; our present results) but not in rats (FUENTES and LONGO, 1971). Therefore, the following considerations apply to the mouse only.

The carbomethoxy group clearly has a negative influence on tremorigenic activity, which cannot be attributed to changed pharmacokinetics. Furthermore, our results are in full accord with those previously obtained after subcutaneous and intravenous application, indicating that a methoxy group in the indole nucleus enhances tremor producing potency whereas an hydroxy group somewhere in the molecule has a diminishing effect (ZETLER,

1964; ZETLER *et al.*, 1972; SINGBARTL *et al.*, 1972). These findings strongly support the view that specific drug receptors for indole derivatives do exist in brain structures important for generation of tremor. The red nucleus and substantia nigra are supposed to be brain parts essential in this respect (POIRIER, SOURKES, BOUVIER, BOUCHER and CARABIN, 1966; SOURKES and POIRIER, 1968; COX and POTKONJAK 1971; LAROCHELLE, BÉDARD, POIRIER and SOURKES, 1971). It is interesting to note that the same structure-activity relationships have been found for negative chronotropic actions on the pacemaker of the guinea-pig heart (ZETLER, LENSCHOW and PRENGER-BERNINGHOFF, 1968). Thus, for the discussed actions on both tissues (a) the complicated isoquinuclidine ring can be considered non-critical and (b) the $COOCH_3$ group, by forming an angle of 90° with the plane of the flat indole ring, profoundly disturbs the complementarity between drug and receptor.

Cerebral drug concentrations at the end of tremor after peripheral administration are a true measure of tremorigenic power of a given compound. These concentrations are lower for all alkaloids than those obtained after intracerebral injection. The factor by which both concentrations differ (column 3 of Table 2) should be the same for all drugs if the pharmacokinetic events after intracerebral application were identical. These factors are nearly equal for harmane, harmine and ibogaine but larger for both iboxygaine and ibogaline. Tremorigenic potency is obviously not correlated with these factors which in the case of iboxygaine and ibogaline probably point to diffusional hindrance not existing for harmane, harmine, and ibogaine. This would also explain why—irrespective of the route of administration—latency periods of tremor are longer after iboxygaine and ibogaline than after the other alkaloids. Latency periods were generally longer after intracerebral than after intravenous injection. This probably indicates that the distance of tremorigenic receptors is greater from the point of intracerebral injection than from capillaries (e.g. in rat cortex <30 μm: DIEMER and HENN, 1965). Considering the distribution coefficients does not reveal that lipophilic nature essentially influences any parameter shown in Table 2.

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