in local concentrations of 0·03–0·01 μg (contained in 0·1 ml physiological saline). Thus, bradykinin B proved very active in causing increased capillary permeability in the guinea-pig. Lacking any direct comparison on the very same test object between bB and, for example, MILES' permeability factor (PF/Dil)[6], or the mediators described by SPECTOR and WILLOUGHBY[7], no comparison is yet possible between the permeabilising activity of either substance.

The authors are indebted to Miss E. HEITZ and Miss C. ZUBER for their valuable technical assistance during these experiments.

R. JAQUES and R. MEIER

Pharmazeutische Abteilung der CIBA Aktiengesellschaft, Basel, June 13, 1960.

Zusammenfassung

Die pharmakologischen Effekte (*in vitro* und *in vivo*) eines durch Einwirkung von Schlangengift (*Bothrops jararaca*) auf Rinderplasma erhaltenen, reinen Bradykinins (B) werden beschrieben.

Post Mortem Increase of 5-Hydroxytryptamine in Rat Brain after 5-Hydroxytryptophan Administration

In several animal species injection of 5-hydroxytryptophan (5HTP) causes an increase of 5-hydroxytryptamine (5HT) in the brain which can be enhanced by pretreatment with monoamine oxidase inhibitors, e. g. iproniazid[1–4]. This 5HT accumulation has been attributed to the fact that 5HTP easily penetrates the brain in which decarboxylation occurs. In presence of great amounts of 5HTP the formation of 5HT probably exceeds its catabolism so that the amine accumulates. This is particularly the case when MAO is blocked.

In the present paper it is shown that 5HTP induced increase of 5HT in brain does not occur *in vivo* only but still continues after decapitation.

Methods. Male Wistar rats fasting for 16 h and weighing 60 to 80 g were decapitated 30 min after i. p. injection of 75 mg/kg DL-5HTP. Part of the animals received 100 mg/kg iproniazid (i.p.) $16^1/_2$ h before 5HTP. The brains were homogenized in 0·1 *n* HCl either immediately after killing (shortest intervall 60 sec) or after the following procedures:

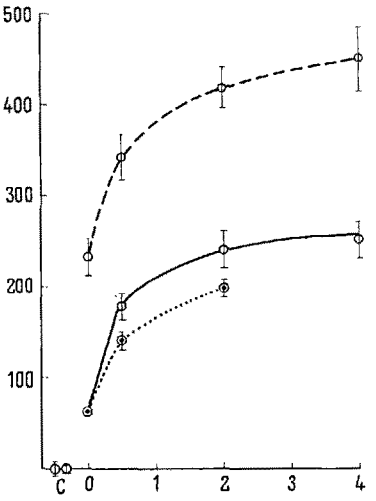
(a) Storage of severed skulls at room temperature and 37°C in closed wet chambers for $^1/_2$ to 4 h.
(b) Storage of whole brains removed immediately after killing in 0·1 *n* HCl at room temperature for 2 h.
(c) Dropping of the skulls into liquid nitrogen immediately after decapitation, removal of the frozen brains in the cold room and homogenization in ice cooled 0·1 *n* HCl.

Animals not treated with 5HTP served as controls. 5HT was determined by a spectrophotofluorometric method[5].

Results. (1) The 5HT content of rat brain was significantly higher after 5HTP injection than in normal animals. The increase of 5HT amounted to 63 ± 5% if the skulls were dropped into liquid nitrogen or to 59 ± 7% in brains taken out at room temperature and homogenized immediately after killing. The difference is not significant ($p > 0{\cdot}05$). Iproniazid pretreatment enhanced this 5HTP induced 5HT increase significantly ($p < 0{\cdot}01$), (Figure, time 0).

(2) If the severed skulls were kept at 37°C before homogenization a further continuous 5HT rise in brain occurred. This was the case in animals with and without iproniazid pretreatment before administration of 5HTP. Storing of the skulls at room temperature had a similar but somewhat less effect (Figure).

(3) Brains of control animals without any treatment kept for 2 h at 37°C showed a significant 5HT decrease during the first half hour as compared with normal brains homogenized immediately after death. In animals only pretreated with iproniazid incubation of the brains for 2 h at 37°C caused no significant change in the 5HT content (Table).



Post mortem increase of 5HT in brain of rats treated with 75 mg/kg 5-hydroxytryptophan i.p. 1/2 h before death.

Abscissa. Time in h after decapitation. 0 time = immediate removal and homogenization after decapitation. *Ordinate.* Percentage 5HT increase as compared with controls (C) not pretreated with 5HTP.

○—○ No treatment with iproniazid. Storage of skulls at 37°C.
○---○ i.p. injection of 100 mg/kg iproniazid $16^1/_2$ h before death. Storage of skulls at 37°C.
○ ··· ○ No iproniazid treatment. Storage of intact brains in 0·1 *n* HCl at room temperature.

Each point represents average of 8–10 single values with standard error. 5 HT of 22 control rats (C) amounted to 0.52 ± 0.02 μg/g fresh weight of brain.

Time *post mortem* (h)	0	1/2	2
No pretreatment	100 ± 4	*80* ± *2*	*82* ± *2*
Iproniazid pretreatment	100 ± 4	98 ± 8	103 ± 8

5 HT content in % of normal brains at various intervals after death. Incubation of intact brain at 37°C. 100 mg/kg iproniazid were administered i. p. 16 h before decapitation. Each figure represents the average value of 6 experiments with standard error. Brains removed immediately after decapitation (time 0) served as controls. The underlined values are significantly different from control values ($p < 0{\cdot}01$).

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(4) Storage of intact brains of 5HTP-treated animals for 2 h in 0·1 *n* HCl at room temperature caused a significant 5HT increase (118 ± 11%) as compared to brains homogenized immediately after death (53 ± 10%; $p < 0{\cdot}01$). This 5HT rise was, however, significantly less than that in brains kept at room temperature but not in HCl (226 ± 22; $p < 0{\cdot}01$).

Discussion. The present results with rat brain frozen or homogenized immediately after decapitation confirm that 5HTP causes a significant 5HT rise which is markedly enhanced by pretreatment with iproniazid. The findings show, however, that storage of the brain at room temperature or at 37°C after death causes a further considerable 5HT increase. This rise cannot even be completely abolished by keeping the intact brain in 0·1 *n* HCl.

In earlier experiments this post mortem rise of 5HT might not have been considered. Thus the reported values for the 5HTP induced increase of 5HT in brain were possibly too high and did not reflect the true content *in vivo*. In order to get more reliable results it is necessary to homogenize the brains in HCl or to freeze the skulls immediately after death.

The 5HT accumulation after death is probably due to continuing decarboxylation of injected 5HTP penetrated into the brain. This agrees with the fact that 5HTP decarboxylase does not require oxygen[6-8]. Monoamine oxidase activity, however, which strongly depends on oxygen tension[9] is probably reduced markedly after decapitation so that the newly formed 5HT mostly accumulates. The slight decrease of 5HT in brain of untreated animals during the first half hour *post mortem* can possibly be attributed to a limited amount of oxygen still present which enables a small MAO activity. Other metabolic pathways for 5HT seem not to be involved since this small disappearance of 5HT is blocked by iproniazid.

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Zusammenfassung

Ratten mit und ohne Iproniazid-Vorbehandlung erhielten 30 min vor Dekapitation 5-Hydroxytryptophan (5HTP) i. p. Das intakte Hirn wurde bis zu 4 h bei 37°C, Zimmertemperatur und in 0·1 *n* HCl aufbewahrt. In allen Fällen ergab sich eine starke postmortale 5-Hydroxytryptamin (5HT)-Vermehrung im Gehirn.

Bei unbehandelten Kontrolltieren zeigte sich postmortal ein leichter 5HT-Abfall im Gehirn, der durch Iproniazid-Vorbehandlung aufgehoben wurde.

A New View on an Old Drug: Pilocarpine

Pilocarpine is one of the oldest parasympathomimetic drugs. Where the action upon the iris[1], the salivary glands, the sweat glands, and intestinal smooth muscle is concerned, pilocarpine shares the action of muscarine and acetylcholine[2]. There are, however, many reports[3] from which it appears that pilocarpine has a more complex mode of action. Especially its action upon the heart and the sacral parasympathetic nerve endings is contradictory to that of muscarine. Moreover, the effect of muscarine on the heart can be abolished by pilocarpine in larger doses[4].

It has been found that progressive elongation of the alkylchain in 2-alkyl-4-(trimethylammonium)methyl-1:3-dioxolane ($RFMe_3$) causes a gradual decrease in the intrinsic activity and consequently a gradual change from parasympathomimetics into parasympatholytics[5]. The intrinsic activity[6], being high (1) for a mimetic, low (0) for a lytic, and intermediate for a partial agonist[7], is a measure for the ratio of agonistic-competitive antagonistic actions. The transition substance $PrFMe_3$ has an intermediate intrinsic activity (0·5) and hence exerts both a parasympathomimetic and a parasympatholytic influence. $PrFMe_3$ is an example of a partial agonist or competitive dualist.

These findings led to the supposition that pilocarpine might be an example of a natural dualistic drug. This implies that pilocarpine would behave both as a parasympathomimetic and a parasympatholytic like $PrFMe_3$. Consequently pilocarpine has to act synergistically with low doses of acetylcholine, but competitive-antagonistically with high doses of ACh.

As a partial agonist, the maximal height of the dose-response curve of pilocarpine on the intestine should necessarily be a fraction of that of acetylcholine. This, as a matter of fact, appeared to be true, as may be seen from Figure 1. The maximal response of pilocarpine is actually about 70% of that of a pure parasympathomimetic such as acetylcholine or furmethonium (HFurf).

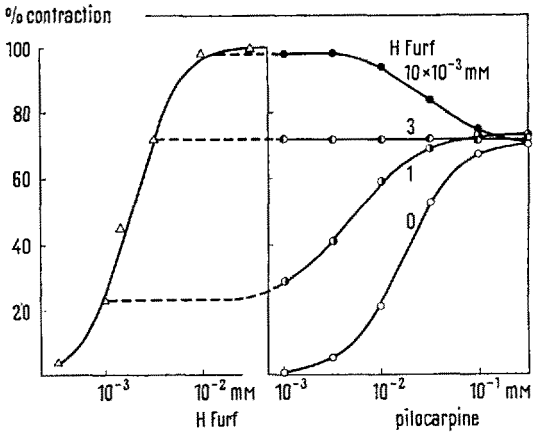

Fig. 1. a) Dose-response curve for the parasympathomimetic furmethonium (HFurf). b) Dose-response curves for the partial agonist pilocarpine in the presence of various doses of HFurf on the isolated gut of the rat.

Note the parasympathomimetic action of pilocarpine in the absence of furmethonium and the competitive antagonistic action when the intestine is brought into contraction by high doses of furmethonium. When a contraction is produced by the agonist equal to the maximal contraction possible with pilocarpine, the latter drug only displaces furmethonium from the receptors without altering the degree of contraction.

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