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Intrahippocampal Injection of Chemicals: Analysis of Spread[1]

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OTT, T., M. SCHMITT, M. KRUG AND H. MATTHIES. *Intrahippocampal injection of chemicals: analysis of spread.* PHARMAC. BIOCHEM. BEHAV. 2(6) 715–718, 1974. – Using intrahippocampal injection as an example, the present paper describes the spreading of solutions of chemicals in the brain after topical cerebral injection (TCI). Detection of radioactivity in different brain regions after intrahippocampal injection of ^{3}H-orotic acid revealed a distribution pattern that cannot be explained by radial diffusion of the substance from the site of injection. The EEG flattening induced by application of KCl (spreading depression) was used as an additional index of the distribution of a chemical after injection into the hippocampus. The biochemical and electrophysiological experiments showed two ways of spread to be of importance to the distribution pattern under the experimental conditions chosen: (a) spreading by transport up the outside of the implanted microcannula, and (b) flow back of the substance into the ventricular system with subsequent diffusion into adjacent brain structures. This type of distribution is almost independent of the physicochemical properties of the substance injected. Therefore, when using TCI, it should be taken into consideration the the substance might spread into other brain areas.

Topical cerebral injection Brain Distribution Hippocampus Cortex Ventricular system Hippocampal injection

TOPICAL cerebral injection (TCI) of dissolved chemicals has become as essential method in solving many neuropharmacological and behavioral problems [1, 7, 10, 15]. As distinct from electrical stimulation, the use of TCI enables definite brain areas to be chemically influenced in quite a selective manner [9,10]. However, TCI is disadvantageous in that spreading of the dissolved substance into other brain regions can frequently not be supervised.

Spreading of the substance can be restricted to a few millimeters by implantation of crystalls [6,8]. However, the value of this method is limited considerably by a number of disadvantages (for critical review see [15]).

When compared with the frequency of their use, studies on the spreading of dissolved chemicals after TCI are not often found in literature. Therefore, difficulties are experienced in interpreting results obtained using this method.

Using intrahippocampal injection, this investigation deals with quantitative and qualitative distribution of a dissolved substance in the brain after TCI. Biochemical experiments were used to determine radioactivity in different brain regions after intrahippocampal injection of labeled orotic acid, while in EEG experiments use was made of the characteristic change in bioelectrical activity induced by intrahippocampal application of KCl [2,3] as a very sensitive parameter of KCl spreading.

METHOD

Animals

A total of 21 male Wistar rats weighing between 180 and 220 g were used throughout the experiments.

Apparatus

Microcannula. The microcannula used (Fig. 1) consisted of a brass body with conical bore in which a cannula wire of 0.65 mm o.d. was a press-fit. To prevent flow-back of the injected solution, the upper orifice of the cannula body was covered by a thin rubber plate flanged into the cannula body. In EEG-studies, an electrode was fitted on the microcannula enabling EEG recording at the site of injection. For this purpose, slits were cut in the mounting flange of the cannula body and a groove recessed in its underside. This groove was fitted with a 0.2 mm thick teflon insulated steel wire, which was cemented to the cannula body by means of Epasol®. The recording wire was soldered to a 1.0 mm dia. silver pin on which the recording cable was fixed via a plug contact.

Procedure

Implantation of cannulae. The skull was exposed under

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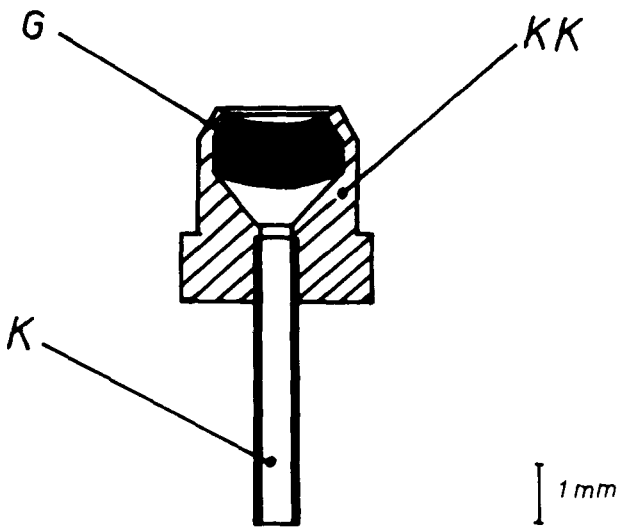

FIG. 1. Microcannula. K: cannula; Kk: cannula body; G: thin rubber plate.

anaesthesia with 600 mg/kg Urethan (VEB Philopharm) and 100 mg/kg hexobarbital. The microcannulae were stereotaxically introduced bilaterally to a depth of 3.2 mm through a hole above the dorsal hippocampus according to the coordinates AP 3.2 mm. lateral 2.5 mm [5] and fixed in this position using cement. As required, additional silver pins were implanted in the same way as surface electrodes. Upon completion of surgical treatment, the animals were allowed to stay in individual cages for one week.

Injection procedure. The solutions were injected using a 10 μl Hamilton microsyringe via a teflon tube with an injection needle (27 ga) fitted. The injection needle was advanced through the rubber plate to the bottom edge of the implanted microcannula. One μl of the particular solution was injected within 10 sec. The injection needle was allowed to remain in this position for another 3 sec and then carefully withdrawn.

EEG recording. The rats were anaesthetized (Hexobarbital-Urethan) one week after implantation of the microcannula and the electrodes. After 30 min of control recording of hippocampal and cortical EEG, 1 μl of 25 percent KCl solution was injected into the left dorsal hippocampus in the manner described above. It was approximately 20 sec later that the EEG could be recorded again. Unipolar recording was from both the left dorsal hippocampus and the left frontal cortex against an indifferent electrode located frontally on the right.

Radiochemical determination of districution. Tritiated orotic acid (1 c/nmol, Radiochemical Centre Amersham), dissolved in artifical cerebrospinal fluid, was used in these experiments. One μl each of this solution was injected into the left and right dorsal hippocampus, respectively. The rats were decapitated 5 or 60 min later and the brains removed within 60 sec. The dissection technique to obtain the individual regions, i.e. frontal cortex (FC), visual cortex (VC), auditory cortex (AC), dorsal hippocampus (DH), ventral hippocampus (VH), thalamus (TH) and brain stem (BS), was in accordance with the method described by Popov *et al.* [13]. Tissue was weighed and frozen until analyses.

Each region was homogenized in 5 ml of ice cold isotonic saline using a motor driven Potter homogenizer. Two aliquots of 0.5 ml each were taken from the whole homogenate, 0.7 ml each of 1 M hyamine hydroxide (Packard) added, and incubated at 60° C for 24 hr. One volume of ice cold 14% trichloroacetic acid was added to the rest of the homogenate. After a short homogenisation it was centrifuged at 5000 g (av.) for 10 min. Fourteen ml each of a dioxane scintillator were added both to 1 ml aliquots of acid-soluble supernatant and whole homogenate samples, respectively. Radioactivity was measured on a liquid scintillation spectrometer provided with automatic quenching correction. Results were expressed as dpm/mg tissue (wet weight).

RESULTS

Biochemical Experiments

When compared with the total activity of the injected volume of ^{3}H-orotic acid, only 26.9 and 26.0 percent of the activity were detected in the whole homogenate of the brain regions under investigation 5 and 60 min, respectively, after injection. This loss was due to the fact firstly that not all of the brain regions were examined, secondly because of diffusion of the labeled substance from out of the tissue into the blood, and thirdly, because of the solution being partially retained in the chronic microcannula. No attempts were made to quantitatively investigate the reason for the loss of radioactivity.

The regional distribution of radioactivity 5 min after injection showed the highest concentration of the substance injected to be located at the site of injection, i.e. in dorsal hippocampus (Table 1). In addition, a considerable amount was also found in visual cortex. In comparison, the values obtained for all remaining regions investigated ranged from 1 to 4% (Table 1). This distribution pattern did not undergo any qualitative change during the course of 60 min, i.e. the highest concentration of radioactivity during this time interval was still found in dorsal hippocampus. Visual cortex also revealed a relatively high concentration (Table 1). While the label decreased in these two regions, radioactivity considerably increased in the subcortical areas (ventral hippocampus, thalamus, brain stem) when compared with the values observed after 5 min. However, this increase was statistically significant for the brain stem only ($t = 2.72$; $p<0.05$; Table 1).

EEG Experiments

In order to obtain better evidence for the reason of the relatively high radioactivity found in visual cortex, the cortical EEG was recorded after intrahippocampal KCl injection. Thus provision was made to follow the occurrence of the intrahippocampal injected substance in cortex more exactly in terms of time, when compared with the biochemical experiment, and based on the well known KCl effect (spreading depression). A characteristic result from the series of EEG experiments is shown in Fig. 2. As clearly visible, bioelectrical activity of the dorsal hippocampus had become almost extinct 30 sec after intrahippocampal injection of 1 μl of 25 percent KCl solution. The typical pattern of a hippocampal spreading depression was maintained for some 2 hr. It was not earlier than 4 hr after the application that the hippocampal EEG reached normal values (Fig. 2). Likewise, spreading depression was observed in the EEG

TABLE 1

TOTAL HOMOGENATE RADIOACTIVITY OBTAINED FOR SEVERAL BRAIN REGIONS 5 MIN (A) AND 60 MIN (B) AFTER INJECTION OF 1 μl ^{3}H-OROTIC ACID* PER HIPPOCAMPUS

Region†	Weight (mg)	Homogenate activity dpm/mg tissue‡	Percent
A			
FC	360 ± 29	1178 ± 420	1.26
VC	270 ± 13	22898 ± 4021	24.44
AC	125 ± 2	2691 ± 1026	2.87
DH	61 ± 4	59222 ± 13868	63.20
VH	84 ± 3	3675 ± 1699	3.92
TH	236 ± 17	3220 ± 1858	3.44
BS	304 ± 11	825 ± 373	0.88
B			
FC	373 ± 20	1779 ± 483	2.14
VC	270 ± 6	16353 ± 2523	19.65
AC	122 ± 5	1603 ± 759	1.93
DH	68 ± 7	46858 ± 6330	56.33
VH	72 ± 4	6477 ± 2530	7.78
TH	218 ± 7	6274 ± 2033	7.54
BS	286 ± 11	3863 ± 1054	4.64

*12.5 μCi 5-^{3}H-orotic acid
†For explanation of abbreviations see "Materials and Methods"
‡Mean values of 4 rats ± SEM

simultaneously recorded from the frontal cortex, namely with 2 min latency, but less pronounced and of short duration only (Fig. 2).

DISCUSSION

In recent years, a number of papers have been published dealing with problems of TCI [1, 11, 12, 15, 16, 17]. In a critical review by Routtenberg [15] the method of TCI was shown to be frequently used, although the possibilities and limitations of its convenience have been examined to an insufficient degree only. This is particularly true for the distribution pattern of an topically injected substance.

The present results show the highest concentration of labeled orotic acid to occur at the site of injection, i.e. the dorsal hippocampus. Likewise, a high concentration was found in the visual cortex, while that in other regions under investigation was comparatively low. From these different concentrations, it can be concluded that, in addition to the radial diffusion of the applied substance from the site of injection, there are further factors involved in the formation of the distribution pattern.

One of the factors essential to the distribution pattern can be derived from the hippocampal and cortical electroencephalograms resulting after intrahippocampal injection of KCl. The cortical spreading depression (CSD) observed in the frontal cortex approximately 2 min after hippocampal spreading depression (HSD) can only be explained by the presence of KCl in the cortex. As indicated by Fifkova [4], functional relationships between hippocampus and cortex are not considered as the cause of CSD. Furthermore, this latter assumption is supported by the different times of appearance and disappearance of HSD and CSD (Fig. 2). Therefore, it might be assumed that part of the substance injected into the dorsal hippocampus moves up the outside of the implanted microcannula into the cortex located thereabove. It is there that a SD is locally initiated spreading throughout the cortex [2], which, however, is of short duration only because the duration of SD follows the local concentration of KCl. This assumption is confirmed by the high concentration of orotic acid in visual cortex, though injected into hippocampus (Table 1). This distribution modifying factor, which was already suggested by MacLean [8], appears to be of significance, since it depends on the chemical properties of the injected substance to a minor degree only. The flow-back of the solution into the ventricle above hippocampus and subsequent diffusion into adjacent structures must be taken into consideration as a further possibility to explain the occurrence of an intrahippocampally injected substance in other brain structures [15]. This portion of labeled substance is displaced towards regions adjacent to the ventricular system (ventral hippocampus, thalamus, brain stem), as is clear from a comparison of homogenate activity observed for different brain regions 5 and 60 min after injection. This result can be taken to reflect the flow-back of the substance into the ventricular system with subsequent slow diffusion into adjacent regions. Both spreading paths of the intrahippocampally applied substance are of general importance, since, in quantitative terms, they are likely to play a more essential part than the radial diffusion from the site of injection.

Allowing for the relatively small injection volume (1 μl), it is therefore assumed that qualitatively similar distribution processes would also occur with other methodical versions of TCI. Thus, when interpreting results obtained by means of TCI, the limits of evidence should be taken into consideration, because simultaneous pharmacological influence on brain areas other than the target region are likely to occur. This is particularly true for experiments where use is made of greater injection volumes.

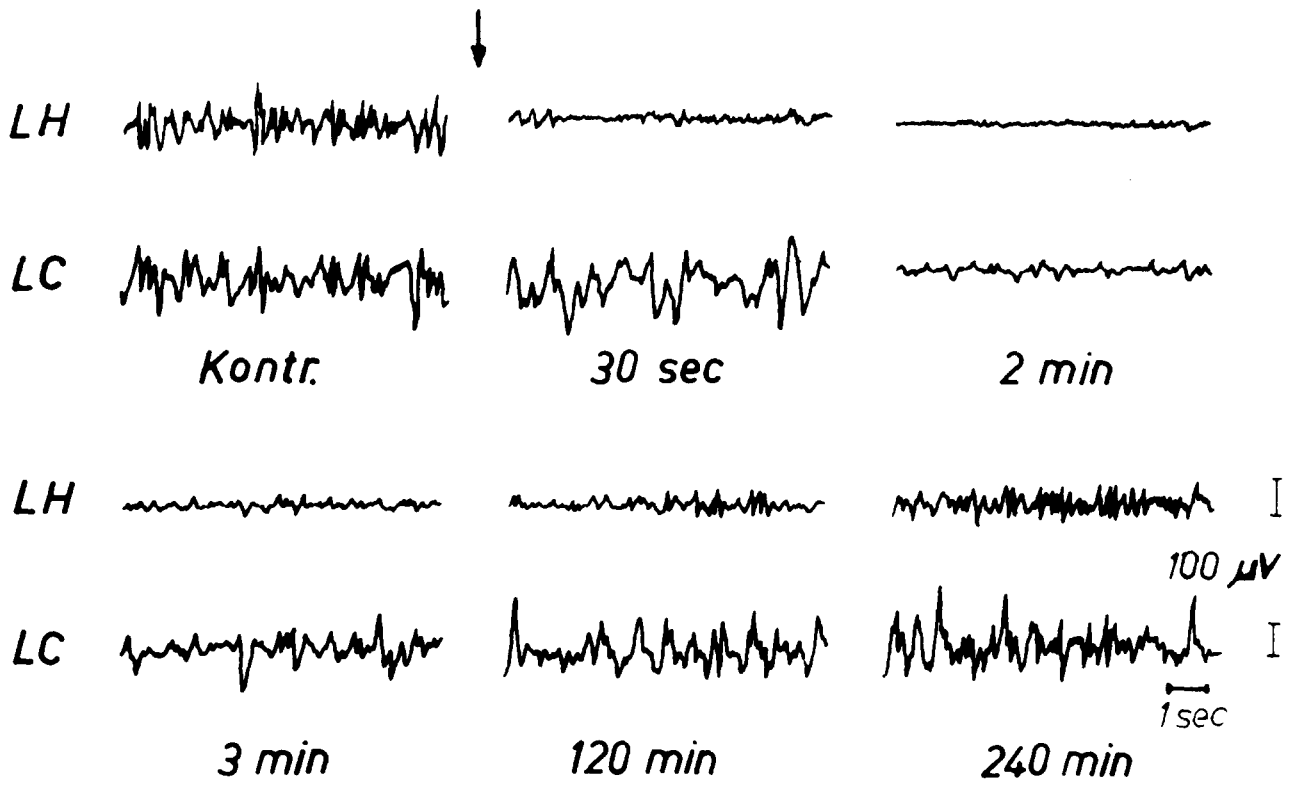

FIG. 2. Recording the EEG of the left dorsal hippocampus (LH) and left frontal cortex (LC) of an anaesthetized rat (experiment 12/72). ↓: Injection of 1 µl of 25 percent KCl solution into the left dorsal hippocampus. Times are relative to injection onset. Ko: Control record 2 min prior to injection.

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