〔Orignal〕

The Relationship Between the Pharmacological Effects of Benzodiazepines and Their in vivo Binding Sites in the Brain of Rats.

Masahiro KOKUBU, Fumio OHTOMO,
Yuichi ENDO, Noboru SHINYA.

Department of Dental Anesthesiology, School of Dentistry,
HIGASHI-NIPPON-GAKUEN UNIVERSITY.

(Chief: Prof, Noboru SHINYA)

Abstract

To compare the in vitro pharmacological effect and in the brain distribution of benzodiazepines and phenobarbital, three group of sixty anesthetized rats (470～480 g) were administered 5 μCi of ^{3}H-diazepam, ^{3}H-flunitrazepam or ^{3}H-phenobarbital. The rats were decapitated 3, 10, or 40 minutes after the intravenous injection of these drugs. Radioactivity of the tissue was measured and calculated as d.p.m./g. tissue. ^{3}H-diazepam radioactivity in the brainstem and hypothalamus was significantly higher than in the brain cortex 3 and 10 minutes after the injection. ^{3}H-flunitrazepam radioactivity in the brain cortex was higher than in other regions. There was no significant decrease in ^{3}H-phenobarbital brain concentration, even 40 minutes after injection. The distribution of benzodiazepine is closely related with its pharmacological effect, and this suggests that in vitro benzodiazepine binding sites are not responsible for the pharmacological action in vivo.

Key words : Benzodiazepines, Brain distribution, Pharmacological effect, Phenobarbital, Radioactivity

Introduction

Benzodiazepines are widely used tranquilizers which are used clinically as anxiolytics, anticonvulsants, sedative-hypnotics and, muscle relaxants. Recent benzodiazepine research has established both findings a specific benzodiazepine receptor in the central nerve system.[1,2,3] and a close relation betweeen benzodiazepine and GABAergic synaptic transmission[4,5].

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Braestrup and his colleagues[2] demonstrated significant correlations between pharmacological activity and affinity to benzodiazepine receptors in vitro.

The distribution of benzodiazepine receptors and GABA receptors in the brain are not identical, especially in the cerebellum[6]. This discrepancy may be due to the GABA receptor heterogenity with some types not associated with benzodiazepine receptors[7,8]. Garattini and his colleagues[9] indicate that benzodiazepines appear to act as muscle relaxants when many benzodiazepine receptors are occupied by other drug. The specific binding of diazepam to the synaptosomal membrane is highest at temperatures of 0～4°C in vitro, but is reduced by more than 95 % at physiological temperature[2] raises the questions whether receptor binding occurs in vivo. Brain benzodiazepine levels found after injections of pharmacological active doses are at least 100～1000 times higher than the concentration which occupies 50 % of benzodiazepine receptor sites in vitro[9,10].

These findings may suggest that the benzodiazepine binding sites detected in vitro are not responsible for the pharmacological action of the drug.

The present study was designed to compare pharmacological effect and brain distribution of benzodiazepines and phenobarbital in vivo.

Materials and Methods

Sixty rats anesthetized with halothane weighing 470 to 580 g were divided into three groups which were administered diazepam (group 1), flunitrazepam (group 2) or phenobarbital (group 3) intravenously through a femoral vein.

Group 1 : thirty rats administered 5 μCi of (N-methyl-^{3}H)-diazepam (specific activity 60 Ci/mmol, Amersham Japan)

Group 2 : fifteen rats administered 5 μCi of (N-methyl-^{3}H)-flunitrazepam (specific activity 79.6 Ci/mmol, Amersham Japan)

Group 3 : fifteen rats administered 5 μCi of phenobarbital (Ethyl-5 phenylbarbituric acid, specific activity 3.7 Ci/mmol NEN)

Animals of each group were decapitated 3, 10 or 40 minutes after the injection. Blood samples were taken, and all tissue was removed immediately. The brains were dissected into the frontal cortex, occipital cortex, lateral cortex, cerebullum, brainstem, hypothalamus, and the "REST". Other organs, liver, heart, kidney, muscle, intestine, and fat were also removed. The tissue was weighed and dissolved with Protosol® by shaking for twenty-four hours at 50 °C. After the tissue was completely solubilized, 0.5 ml of of the tissue solution were neutralized with 0.5 N HCl and 4 ml of liquid scintilation cocktail were added. The radioactivity of the tissue was measured by a liquid scintilation counter for ten minutes. Plasma was separated from the blood and 1 ml was used to measure the radioactivity. All radioactivity levels were calculated as d.p.m / g. tissue.

Results

Table 1 shows the radioactivity following injection of ^{3}H-diazepam, ^{3}H-flunitrazepam, and ^{3}H-phenobarbital. The distribution in the brain is summarized in Figures 1~4. The radioactivity of other organs except liver and fat show similar change, decreases with time. ^{3}H-phenobarbital concentrations are markedly higher than ^{3}H-diazepam and ^{3}H-flunitrazepam at all times.

Table 1 Radioactivity (d.p.m./g. tissue) in the rat Brain, Liver, Heart, Kidney, Muscle, Fat, Intestine and Plasma following administration of 5 μCi ^{3}H-diazepam, ^{3}H-flunitrazepam and ^{3}H-phenobarbital.

Tissue / Drugs and time	Brain								other organs						
	Cortex				Cerebellum	Brainstem	Hypothalamus	"Rest"	Liver	Heart	Kidney	Muscle	Fat	Intestine	Plasma
	frontal	occipital	lateral	mean											
3minutes after administration															
group1(n=10)	65,790	63,414	61,657	63,620	64,374	79,599	89,298	54,730	131,614	106,651	103,717	17,656	11,377	61,373	14,880
group2(n= 5)	101,665	116,571	98,239	105,492	65,869	67,273	78,740	72,409	171,953	108,692	121,538	59,070	25,846	88,259	22,772
group3(n= 5)	97,507	97,231	93,310	95,349	91,220	92,984	89,533	69,343	289,071	204,001	203,164	84,443	76,358	193,551	86,504
10minutes after administration															
group1(n=10)	38,290	37,908	37,181	37,793	35,037	41,718	48,477	29,763	182,565	72,708	81,766	22,147	17,933	57,608	13,418
group2(n= 5)	40,086	42,484	39,198	40,589	30,775	30,466	35,672	29,935	225,566	60,274	91,804	18,026	19,872	55,320	13,165
group3(n= 5)	104,090	109,133	103,683	105,635	99,285	114,097	111,380	84,444	231,671	160,165	161,744	99,688	64,370	152,490	66,639
40minutes after administration															
group1(n=10)	13,571	14,079	12,764	13,471	12,882	17,296	19,743	14,950	174,992	24,333	63,112	11,581	40,392	89,477	9,347
group2(n= 5)	9,809	12,980	10,659	11,149	7,307	11,161	13,763	5,342	48,400	22,629	30,523	11,251	20,149	16,771	2,580
group3(n= 5)	83,000	85,454	76,243	81,567	78,656	89,873	92,412	75,027	187,648	123,942	139,980	91,090	80,814	116,733	53,930

group 1 (^{3}H-diazepam) group 2 (^{3}H-flunitrazepam) group 3 (^{3}H-phenobarbital)

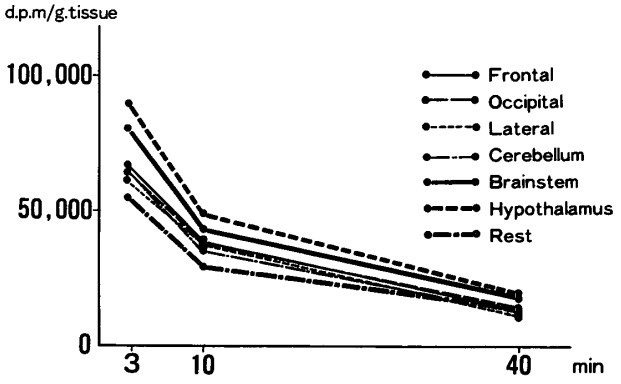

Fig. 1 Brain concentration of ^{3}H-diazepam (d.p.m/g. tissue). Maximum radioactivity was seen at 3 minutes following injection. ^{3}H-diazepam concentration in the brainstem and hypothalamus were higher than in the cortex and others.

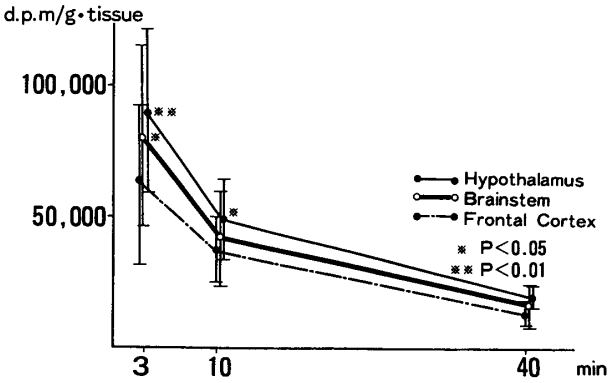

Fig. 2 ^{3}H-diazepam concentrations (d.p.m/g. tissue) were averaged to compare with that of the brainstem and hypothalasmus. ^{3}H-diazepam radioactivity in the brainstem and hypothalamus was significantly higher than in the cortex at 3 and 10 minutes following injection ($p<0.05$ or 0.01, t-test).

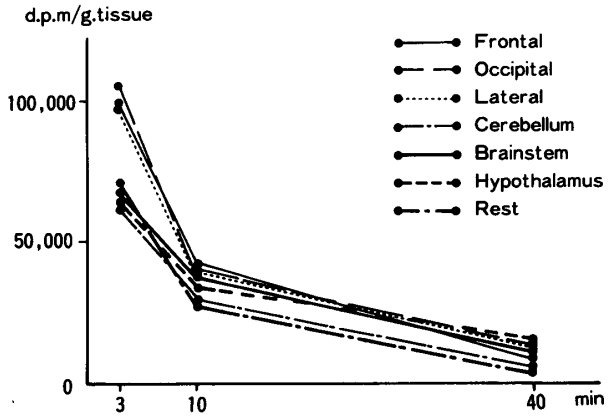

Fig. 3 ^{3}H-flunitrazepam concentration (d.p.m/g. tissue) in the brain of rats. In constract to ^{3}H-diazepam, the concentration in the brain cortex was higher than other brain regions.

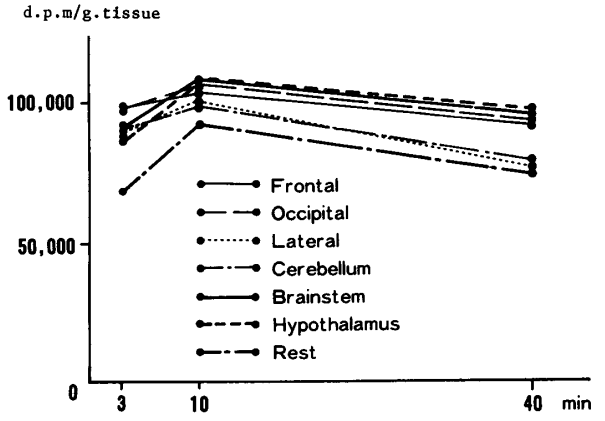

Fig. 4 ^{3}H-phenobarbital concentration in rat brain (d.p.m/g. tissue). A significant decrease of ^{3}H-phenobarbital concentration in rat brain was not seen even at 40 minutes following injection.

Figure 1 shows the brain concentration curves of ^{3}H-diazepam obtained from 10 rats. The maximum radioactivity was reached 3 minutes after the injection. The ^{3}H-diazepam concentration in the hypothalamus and brainstem was higher than in the cortex and other tissue. ^{3}H-diazepam concentration in the frontal, occipital, and lateral cortex were not significantly different. Therefore, these brain cortex concentrations were averaged to compare with the hypothalamus and brainstem (*Fig* 2). The ^{3}H-diazepam radioactivity in the brainstem and hypothalamus were significantly higher than in the cortex at 3 and 10 minutes after the injection ($p<0.05$ or 0.01, t-test).

Figure 3 shows the brain concentration curves of ^{3}H-flunitrazepam obtained from 5 rats. The ^{3}H-flunitrazepam concentration curves were very similar to the ^{3}H-diazepam concentration curves. However, the ^{3}H-flunitrazepam concentration in the brain cortex was higher than in other brain regions, especially at 3 and 10 minutes different from ^{3}H-diazepam.

Figure 4 shows the brain concentration curves of ^{3}H-phenobarbital obtained from 5 rats. The results differ from ^{3}H-diazepam and ^{3}H-flunitrazepam, especially in the rate of decrease between 10 and 40 minutes. There is no significant decrease in ^{3}H-phenobarbital brain concentration even at 40 minutes after the injection.

Discussion

This study has determined that pharmacological activity of benzodiazepines and phenobarbital are closely related with the distribution of these drugs in the brain of rat.

Compared with diazepam, ^{3}H-flunitrazepam was found in high concentrations in the rat brain and this may support the potent hypnotic effect.

The effect of phenobarbital, lasts much longer than that of diazepam and flunitrazepam, and may be due to the longer lasting high concentration in the brain and other organs.

The anticonvulsant effect of diazepam and phenobarbital may be due to their high concentra-

tions in the brainstem and hypothalamus. Tonic and clonic convulsion may be explained by an activation of the thalamo-reticulospinal system[11]. Anticonvulsant drugs are thought to prevent convulsions by depressing the effect of the thalamo-reticulospinal system through three mechanisms: a stabilization of the neuronal membrane, a decrease in the repetitive discharge, and a reduction in the spread of seizure discharges to the cortex neuron at brainstem and thalamus[12].

Mennini and his colleague[13] indicated that there is no simple correlation between receptor occupancy in vitro and the pharmacological effect. However, although the existence of high affinity binding sites for benzodiazepines has been well documented from in vitro studies, it is not possible to extrapolate the mechanism of action to in vivo situations. Moreover, Benzodiazepine binding sites are not identical with the GABA receptor binding sites, as rats GABA agonists and antagonists fail to influence diazepam binding in similar experiments[14].

Our finding, that benzodiazepines distribution is closely related to its pharmacological effects, suggests that the benzodiazepine binding sites determined in vitro are not responsible for the pharmacological activity in vivo.

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ベンゾディアゼピン系薬剤の薬理作用と脳内結合部位との関連について

國分　正廣，大友　文夫，遠藤　祐一，新家　昇

東日本学園大学歯学部歯科麻酔学講座

（主任：新家　昇教授）

和文抄録

ベンゾジアゼピン系薬剤とフェノバルビタール薬理作用と脳内分布との関連を生体内で調べるため，60匹のラット（体重470～480 g）を3郡に分け，^{3}H-diazepam，^{3}H-flunitrazepam または^{3}H-phenobarbital 5 μciを投与した。それぞれのグループのラットは薬剤の静脈内投与後3分，10分，または40分の時点で断頭し，各組織の放射能を測定し，d.p.m/g•tissueに換算した。この結果，^{3}H-diazepamの放射線量は脳幹および視床下部で他の大脳皮質に比べ有意に高かった。また，^{3}H-phenobarbitalの脳内濃度は換与後40分経ってもほとんど低下しなかった。これらの結果はベンゾジアゼピン系薬剤の薬理作用との密接な関連があり，in-vitroで証明されたbenzobiazepine receptorはin-vivoではあまり重要な意味を持たない可能性があることを示唆している。

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