SYNTHESIS, CHROMATOGRAPHY AND TISSUE DISTRIBUTION OF 1-[^{11}C]-HEXOBARBITAL

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SUMMARY

1-[^{11}C]-Hexobarbital was synthetized by methylation of norhexobarbital using $^{11}CH_3I$. The maximum radiochemical yield after purification by hplc is 47% at a specific activity of 1.0 mCi/μmole. Synthesis time including purification is 37 min. The tissue distribution was determined in mice at different times after i.v. injection. The main accumulation of activity during the first hour after i.v. injection is in liver and kidneys. Other well perfused organs like brain, heart and lung show significant accumulation only during the first minutes after injection.

Key words: Carbon-11, Hexobarbital, Tissue distribution

INTRODUCTION

Short-acting barbiturates, like hexobarbital and thiopental, are widely used as centrally acting anaesthetics. They attain a high concentration in the central nervous system shortly after i.v. injection (1); thus the anaesthetic state is reached without an excitation phase as intermediate. These highly lipophilic drugs easily cross the blood-brain barrier. Their action ceases shortly after i.v. injection. This rapid termination of activity is not due to an extremely rapid metabolism of hexobarbital but to redistribution within the body via circulation. The normally perfused tissues like lean body mass and muscles act as a sink (2). The metabolism of hexobarbital is relatively slow; the main metabolic products are ketohexobarbital, norhexobarbital and keto-norhexobarbital, the ketone function being introduced in the cyclohexenyl ring (3,4,5,8). Among these products ketohexobarbital is the most

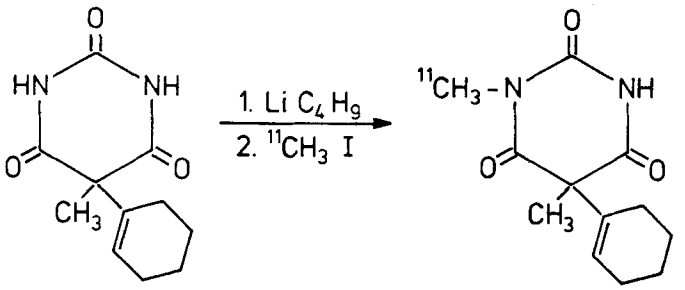
0362-4803/81/050731-08$01.00

Received February 12, 1980

abundant. The N-demethylated compounds, in contrast, are only of minor importance. We felt that these data make hexobarbital a useful tracer for cerebral blood flow when labelled with a suitable short-lived radionuclide. The positron emitting nuclide carbon-11 ($T_{1/2}$ = 20.3 min) is suitable for this purpose, since it has short half-life and makes regional cerebral blood flow measurements possible using positron emission tomography.
We decided to label hexobarbital in the N-methyl-group, since this position can be labelled rather conveniently by $^{11}CH_3I$. The label will not easily be lost, since metabolism is rather slow and N-demethylated metabolites are only of minor importance (5).

EXPERIMENTAL

Norhexobarbital was a generous gift of Bayer AG, Leverkusen, FRG.
Practically carrier-free $^{11}CH_3I$ was prepared according to Marazano et al. (6).



The alkylation was a modified procedure according to Wang (7). A typical preparation of 1-[^{11}C]-hexobarbital runs as follows:

> To 18 mg of norhexobarbital in 2 ml dimethylformamide, 30 ml of a 2N solution of n-butyl-lithium in n-hexane was added. This mixture was left for 20-30 min at room temperature.

t = 0 min — Practically carrier-free $^{11}CH_3I$ (8.0 mCi) was transferred into this solution with a stream of helium gas. The mixture was stirred at room temperature for 10 min. 1 ml of water was added; the resulting solution was evaporated to dryness using an oil-pump.

t = 15 min — The residue was taken up in 1 ml of 1N NaOH; this solution was extracted with diethylether (3x0.5 ml). The aqueous phase was acidified with 1.1 ml of 1N HCl and extracted with chloroform (3x0.5 ml). After separation of the phases, the chloroform solution was dried using a minimal amount of Na_2SO_4.

t = 29 min — The chloroform phase is injected onto a hplc-column (LiChroSorb Si60, 10 µ, 50x0.4 cm) via a sample valve. At a flow rate of 4 ml/min using chloroform as eluent, 1-[^{11}C]-hexobarbital elutes with a retention time of 3 min.

t = 33 min — The 1-[^{11}C]-hexobarbital peak is collected and

t = 37 min — evaporated to dryness using a rotary evaporator.

An optimum radiochemical yield of 47% ≙ 3.75 mCi (decay corrected) was obtained with a specific activity of 1.0 mCi/µmole. The range of yields was 25-47% and the total preparation time varies between 30 and 60 min.

It is mandatory to use only norhexobarbital freshly recrystallized from benzene, since norhexobarbital rapidly deteriorates on standing. Low yields are the consequence of using old starting materials. During preparation of $^{11}CH_3I$, about 0.3-1.0 µmole of nonradioactive CH_3I are generated, probably from atmospheric CO_2; therefore, hexobarbital is not carrier-free.

The hplc conditions (Table I) yield a salt-free residue of 1-[^{11}C]-hexobarbital; thus, no problems with the osmolarity of the

injection solution are encountered.

Table I. Chromatographic Data*

compound	k'
hexobarbital	1.5
norhexobarbital	8.75

*column: LiChroSorb Si60, 10µ, 50x0.4 cm
eluent: $CHCl_3$
flow : 4 ml/min

For administration to mice, 1-[^{11}C]-hexobarbital was taken up in 1-5 ml isotonic saline and filtered through a 0.2 µm Millipore filter to yield a sterile solution suitable for injection. Up to now, a maximum of 1 mCi of 1-[^{11}C]-hexobarbital was obtained in injectable solution.

ANIMAL EXPERIMENTS

Throughout the study female NMRI albino mice with body weights ranging from 27 to 33 g were used. 1-10 µCi of 1-[^{11}C]-hexobarbital in 100 µl isotonic saline were injected into the tail vein of the animals confined to a restriction cage. Animals were killed by cervical dislocation after the appropriate time intervals. The organs of interest were removed, blotted dry, counted in a well-type counter using a NaI(Tl)-scintillation detector, and weighed.

Since there was a rather large variation in body weight of the animals, the results are expressed as % mean body concentration (%MBC) in various organs; the %MBC is obtained by dividing

specific activity of the organs (cpm/g) by the applied dose (cpm/g body weight) and multiplying by 100.

DISCUSSION

The pharmacokinetics of hexobarbital are rather well documented in the literature (1,8,9,10,11). These data, however, are mainly concerned with the metabolism and elimination phase of hexobarbital, both of which are only important after the pharmacological effect of hexobarbital, namely narcosis, has ceased. The narcotic effect of hexobarbital after an i.v. dose of 25 mg/kg in rats lasts for about 10 min (1), whereas the metabolism and excretion phase will last for several hours.

Our data cover the period of the pharmacological effect; during this time we expect a very early maximum of accumulation in the brain. Indeed, the maximum accumulation of activity in the brain of mice is reached in less than 0.5 min; at 0.75 min, the brain concentration is 144 %MBC, while the brain-to-blood concentration ratio is nearly 1. Afterwards, the brain concentration falls continuo ly, while the blood concentration remains rather constant. At 3 min p.i., the brain concentration is 84 %MBC, while the brain-to-blood concentration ratio is 0.56. In contrast to our data in mice, literature data in rats (1) show that the maximum brain concentration ratio is reached 3 min p.i. at 220 %MBC, while the brain-to-blood concentration ratio is about 2. The difference in the time of maximum accumulation in the brain can be explained by the different heart rates in both species, whereas we have no explanation for the difference in the brain-to-blood concentration ratio in both species.

All other well perfused organs, such as heart, lung, liver and kidneys concentrate hexobarbital almost as rapidly as the brain. Heart and lung release hexobarbital during the same time interval

Table II. Tissue Distribution of 1-[^{11}C]-Hexobarbital at Different Times after I.V. Injection (n = Number of Animals)

	Organ Investigated							
Time (min)	brain	blood	liver	intestine	kidneys	heart	lung	fat
0.5 (n=5)	146±12	174± 21	90± 25	64± 20	223± 38	236± 13	269± 21	-
0.75(n=8)	144±16	159± 37	120± 40	64± 20	211± 49	195± 34	240± 49	-
1.0 (n=8)	129±22	142± 26	229± 45	87± 22	189± 56	173± 31	218± 39	33± 12
1.25(n=9)	122±24	145± 30	270± 89	85± 12	179± 27	156± 25	195± 35	29± 3
1.5 (n=8)	103±20	113± 19	204± 58	73± 14	165± 55	132± 21	167± 31	-
2.0 (n=6)	97±20	158± 24	285± 30	99± 6	217± 42	178± 55	173± 23	72± 38
2.5 (n=5)	98±20	123± 13	238± 25	84± 4	200± 30	146± 18	160± 17	-
3.0 (n=4)	84±16	149± 21	291± 14	94± 7	193± 33	145± 3	182± 11	-
3.5 (n=4)	64± 9	110± 13	231± 58	78± 16	205± 84	122± 15	142± 19	-
4.0 (n=3)	48-75	85-112	203-257	71- 91	156-264	96-122	138-139	-
4.5 (n=3)	53-56	124-126	234-269	93- 98	222-347	117-126	116-150	-
5.0 (n=3)	41-90	123-182	265-337	96-151	190-392	118-186	121-247	65- 75
10.0 (n=3)	45-54	123-145	175-225	85-110	260-355	113-150	147-190	50-153
30.0 (n=5)	26± 8	95± 7	169± 8	113± 10	365± 48	77± 6	92± 3	71± 24
60.0 (n=4)	13± 5	34± 9	58± 20	87± 12	131± 16	31± 8	32± 13	31± 25

Values expressed as %MBC (see text)

Values are mean ± S.D. for 4 and more animals; for 3 animals only the range is tabulated.

as the brain; only liver and kidneys, the organs of metabolism and excretion, retain the activity for significantly longer time intervals. During the whole period of investigation significant amounts of activity are present in blood. After a redistribution phase, hexobarbital or its metabolites are accumulated in body fat. These data support the theory of Price et al. (2), that short-acting barbiturates, like hexobarbital and thiopental, are rapidly transported to the well-perfused organs; after a short time, they are redistributed via the circulation, the muscle mass and skin acting as a first sink, the body fat being the final sink before metabolism and excretion.

1-[^{11}C]-Hexobarbital may be a useful tracer for regional cerebral blood flow during the first minutes after i.v. injection. It remains to be seen whether this compound shows useful results in other animal species and, finally, in man.

ACKNOWLEDGEMENTS

We thank Prof. Stöcklin for his constant support and stimulating discussions. We also thank W. Wutz and P. Laufer for their valuable technical assistance.

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