^{11}C-Labeled 4-Isopropylantipyrine: Preparation and Biological Evaluation as a Blood Flow Tracer in Positron Emission Tomography (PET)

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Radiolabeled 4-isopropylantipyrine (1) has been synthesized and evaluated as a tracer for the measurement of cerebral blood flow (CBF). Methylation of 4-isopropyl-3-methyl-1-phenylpyrazol-5-one (2) with [^{14}C]methyl iodide in acetonitrile gave [^{14}C]-1 in radiochemical yields of 10–20%. Its blood–brain partition coefficient in rats was determined to be 0.62 ± 0.03 (mean ± SE). Autoradiographic determination of regional cerebral blood flow under normal flow conditions indicated that [^{14}C]-1 gives results essentially identical with those obtained with the widely used tracer [^{14}C]-4-iodoantipyrine ([^{14}C]-IAP). Studies performed in high-flow states indicated that [^{14}C]-1 is not more diffusion limited than [^{14}C]-IAP. A rapid synthesis was therefore developed for the preparation of [^{11}C]-1. Radiochemical yields were increased to 40–50% when the alkylation of 2 with [^{11}C]methyl iodide was performed in dimethyl sulfoxide using solid potassium hydroxide as a base. Since the ^{11}C-labeled compound can easily be produced in large quantities and since the tracer is not diffusion limited at flow rates commonly observed in normal and most pathological states in man, [^{11}C]-4-isopropylantipyrine will be used for in vivo studies of CBF using positron emission tomography.

The investigation of many in vivo processes has been made possible by the development of positron emission tomography (PET). The measurement of regional cerebral blood flow (CBF) in humans has, however, been somewhat delayed by both the method of evaluating the data and the difficulty in finding the ideal tracer. Direct measurements based on dynamic tracer analysis require data sampling times of 1 s or less. Most PET units currently in operation, however, require much longer data acquisition times. To overcome this problem, equilibrium imaging is used. Three major methods that have been proposed for the in vivo study of regional CBF are the tissue-clearance, continuous-inhalation, and the microsphere or tissue-trapping methods. While each method has advantages and disadvantages,[1] the tissue-clearance method has been used more widely than the other techniques.

Ideally, the tracer used in the tissue-clearance method should be freely diffusible, have a known partition coefficient that is invariable for both normal and abnormal cerebral states, be inert under the measurement period, and have no recirculation or one that can be described. Tracers labeled with positron-emitting isotopes such as $H_2{}^{15}O$, ^{77}Kr, [^{11}C]alcohols, [^{18}F]fluoromethane, and a series of antipyrine derivatives have been reviewed.[1] In this paper we present the synthesis of [^{11}C]-4-isopropylantipyrine (1) and the determination in rats of the blood–brain partition coefficient and cerebral blood flow in normal flow state (normocapnia) and a high flow state (hypercapnia).

Selection of the Tracer. Antipyrine is a lipophilic compound that is highly extracted by the brain. However, when the ^{14}C-labeled compound was investigated as a possible tracer for measuring CBF by autoradiographic methods, its uptake was found to be diffusion limited[2] to an extent that invalidated the calculation of CBF. Antipyrine has been labeled with ^{11}C by van Haver et al.,[3] but no reports have, to our knowledge, been published on its use as a CBF tracer.

The lipophilic compound 4-iodoantipyrine (IAP) has a much higher partition coefficient than antipyrine. The ^{14}C-labeled compound has been widely used for measuring CBF autoradiographically in animals.[4] The ^{11}C-labeled IAP has been synthesized by Campbell et al.[5] It has, however, found limited use in PET studies[6] due to the possible contamination by ^{11}C-labeled antipyrine, the difficulty in scaling up the reaction, and the metabolic loss of significant amounts of iodine within 10 min after injection.

Antipyrine has also been labeled with ^{18}F in the 4-position of the pyrazolone ring by Shiue and Wolf.[7] The carbon-fluorine bond is strong, so there is probably little chance of losing the label during the PET investigation. However, since the C–F bond is so similar to the C–H bond, the lipophilicity of 4-fluoroantipyrine might be expected to be more similar to that of antipyrine than to that of IAP. A report on its use as a blood flow tracer in animals has recently been published.[8]

When short-lived isotopes are used in serial PET studies, the second investigation is performed after the level of radioactivity from the previous investigation is essentially 0 or can be accounted for. Injection of a ^{18}F-labeled compound ($t_{1/2}$ = 110 min) generally requires that the second investigation be performed on the succeeding day, while two investigations with ^{11}C-labeled tracers ($t_{1/2}$ = 20 min) can be carried out 2–3 h apart. Since studies of blood flow are often combined with the investigation of another physiological parameter such as metabolism, an appropriate ^{11}C-labeled tracer is preferred. Therefore, our aim was to synthesize a derivative of antipyrine, with the lipophilic characteristics of IAP, that would be stable in vivo during a PET study.

For that purpose, the lipophilic contributions,[9] π*, of the following substituents in an aromatic ring were compared: –H (0.00), –F (0.14), –I (1.12), $-CH_3$ (0.56), $-CH(CH_3)_2$ (1.53). The contribution of two methyl groups would equal

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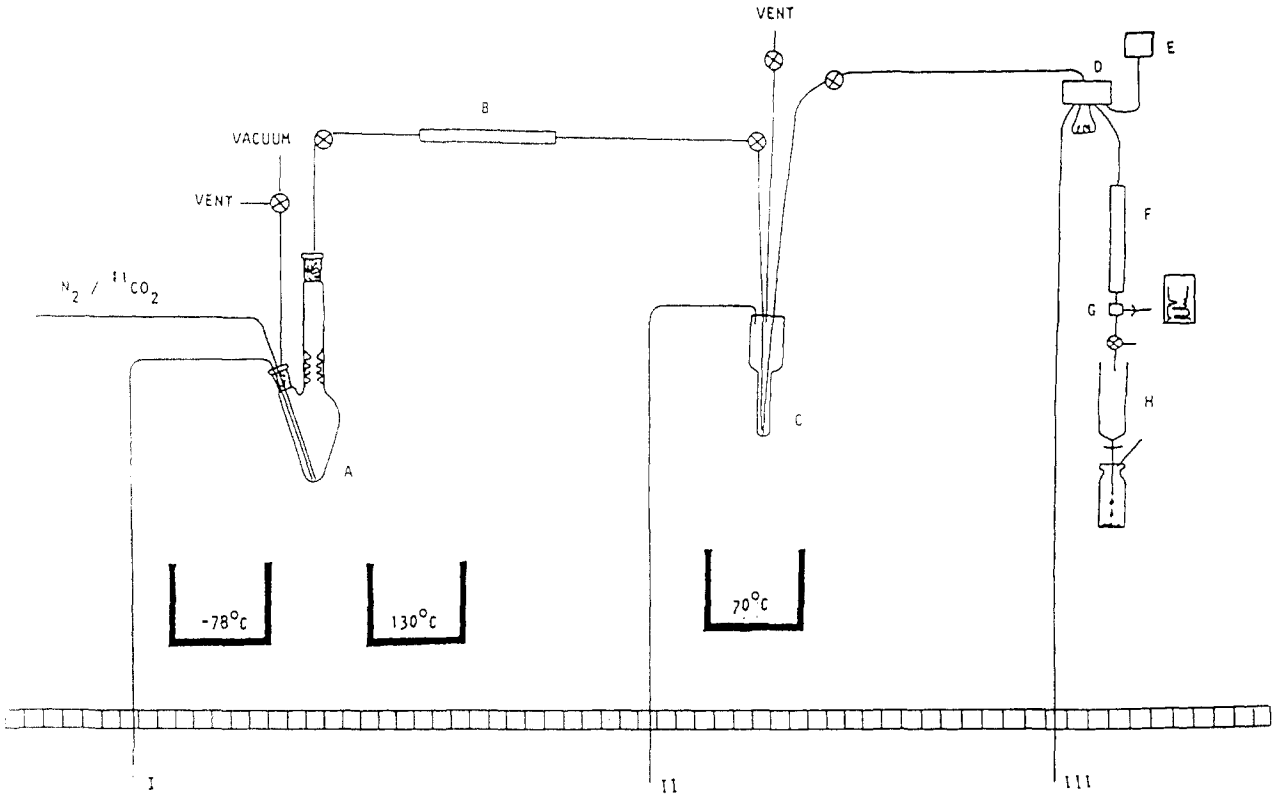


Figure 1. Radiolabeling of 4-isopropylantipyrine with $^{11}CH_3I$: (A) 10 μmol of $LiAlH_4$ in 0.5 mL of THF; (B) soda lime and phosphorus pentoxide trap; (C) 200 μL of Me_2SO + 1–2 grains of solid KOH; (D) Rheodyne injector; (E) LDC Constametric I pump; (F) μ-Bondapak C-18 column; (G) LDC Spectromonitor II and GM tube; (H) Syringe and sterile injection vial for collection of the sample. Syringes for adding: (I) 1 mL of 54% HI; (II) 1 mg of precursor (**2**) + 0.1 mL of Me_2SO; (III) suction.

that of the iodine substituent. However, since the incorporation of an isopropyl group in position 4 of the pyrazolone ring would give propyphenazone (**1**), a compound used in clinical practice as an analgesic,[10] we decided that the radiolabeling of this molecule would be our primary goal.

Results

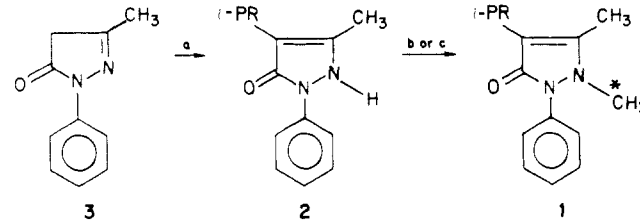
Chemistry. The starting material for the labeling experiments, 4-isopropyl-3-methyl-1-phenylpyrazol-5-one (**2**), was easily prepared from commercially available **3**, as is shown in Scheme I. Yields were good, and **2** is stable as long as it is stored cold and protected from light.

$^{11}CH_3I$ was prepared by a standard procedure in which $^{11}CO_2$ is trapped in a solution of $LiAlH_4$ in THF, the solvent evaporated, and the product distilled from refluxing HI. The yields are high and the process rapid. Higher specific activities may be obtained by using low concentrations of $LiAlH_4$ in a minimum amount of solvent. However, high specific activities are not a prerequisite for CBF measurements, and the presence of unlabeled 4-isopropylantipyrine in the final solution need not be minimized since **1** has been used pharmaceutically in much larger quantities without side effects.

Radiolabeled 4-isopropylantipyrine (**1**) was prepared as depicted in Scheme I. Labeling with $^{14}CH_3I$ was accomplished by the procedure of van Haver et al.[3] Since small volumes were heated at high temperatures, an HPLC valve with a 500-μL loop was used to prevent evaporation of the solvent and $^{14}CH_3I$. Yields obtained varied between 10 and 20% after a 15-min reaction time.

For the ^{11}C labeling a faster, more efficient method of labeling with $^{11}CH_3I$ was developed with solid potassium

Scheme I. Synthesis of 4-Isopropylantipyrine[a]



[a] Key: (a) acetone + methanol, 100 °C, pressure, RaNi; (b) $^{14}CH_3I$, acetonitrile, 170 °C; (c) $^{11}CH_3I$, Me_2SO, KOH, 70 °C.

hydroxide as a base in dimethyl sulfoxide.[11] Labeling yields increased to 40–50%. The reaction time was decreased to 5 min, which also increased the amount of [^{11}C]-**1** obtained by a factor of 1.4 due to the short half-life of ^{11}C. [^{11}C]-**1** was separated from other labeled byproducts by preparative reversed-phase HPLC. The method is rapid and easily reproducible, and the specific activity of the obtained product can be determined by simultaneous measurement of the UV absorption at 241 nm. Dissolution of the evaporated fraction in a solution of ethanol–propylene glycol–physiological saline (1.5:3.5:5) was greater than 99%, and the solution obtained after filtration through a Millipore filter was sterile and free from pyrogens. Total synthesis time of 40 min enables facile preparation of sufficient quantities of [^{11}C]-**1** in vivo studies of cerebral blood flow. The experimental setup for the ^{11}C labeling is shown in Figure 1.

Biological Studies. The partition coefficient of 4-isopropylantipyrine between blood and brain tissue was determined by the procedure described by Abdul-Rahman

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Table I. Comparison of Regional Cerebral Blood Flow in Rats Obtained with 4-Isopropylantipyrine and 4-Iodoantipyrine[13a]

	normocapnia		hypercapnia	
	IpAP* $N = 3$	IAP§ $N = 7$	IpAP* $N = 2$	IAP§ $N = 6$
frontal cortex	2.26	2.03 ± 0.08	5.34	4.67 ± 0.29
caudoputamen	1.35	1.41 ± 0.09	4.18	3.77 ± 0.24
globus pallidus	0.73	0.66 ± 0.07	2.85	2.61 ± 0.20
substantia nigra	1.00	1.01 ± 0.07	3.90	3.21 ± 0.23
nucleus ruber	1.36	1.45 ± 0.10	4.91	3.49 ± 0.22
nucleus accumbens	1.45	1.50 ± 0.10	5.49	4.18 ± 0.37
cerebellum	0.83	0.82 ± 0.06	1.98	2.70 ± 0.33
sensorimotor cortex	1.91	1.98 ± 0.10	7.24	5.75 ± 0.50
parietal cortex	1.89	1.89 ± 0.09	6.95	5.50 ± 0.36
thalamus	1.73	1.63 ± 0.09	6.84	4.77 ± 0.30
visual cortex	1.30	1.52 ± 0.08	4.97	4.08 ± 0.26
lateral geniculate body	1.45	1.40 ± 0.11	5.46	3.97 ± 0.27
superior colliculus	1.49	1.48 ± 0.11	4.66	3.78 ± 0.22
auditory cortex	2.36	3.12 ± 0.15	5.66	5.79 ± 0.28
medial geniculate body	2.18	1.82 ± 0.09	6.94	5.18 ± 0.37
inferior colliculus	1.99	1.99 ± 0.09	5.30	4.76 ± 0.22
piriform cortex	1.05	1.12 ± 0.10	3.56	3.11 ± 0.12
cingulate cortex	1.34	1.33 ± 0.17	3.45	3.90 ± 0.26
hippocampus	0.87	0.87 ± 0.07	2.77	2.84 ± 0.27
amygdala	0.95	1.06 ± 0.09	3.65	3.09 ± 0.10
septal nucleus	0.91	0.85 ± 0.06	3.34	3.21 ± 0.26
habenula	1.43	1.64 ± 0.12	5.29	5.23 ± 0.44
hypothalamus	0.97	1.05 ± 0.08	3.63	3.14 ± 0.21

[a] Local CBF values are means (*) and means ± SE (§) in mL·100 g^{-1}·min^{-1} from measurements made in the number of animals (N) indicated in the headings.

et al.[12] using the ^{14}C-labeled compound. Four animals were used in the experiment. The partition coefficient was found to be 0.62 ± 0.03 (mean ± SE). The corresponding value for IAP[4] is 0.80 ± 0.01.

Cerebral blood flow was measured as described in the Experimental Section. Values for 23 structures were calculated from the partition coefficient given above. The results, compared with those obtained using [^{14}C]-IAP as a tracer under the same experimental conditions,[13] are presented in Table I. In normal flow states, a high degree of correlation was observed between the blood flow values obtained for [^{14}C]-1 and for [^{14}C]-IAP: a linear regression analysis of the two data sets gave $r = 0.93$, and the slope of the curve was close to unity ($y = 1.03x - 0.01$). Furthermore, two animals were studied at the end of a 15-min period of hypercapnia (P_{CO_2} = 78 and 70 mmHg, respectively). Here, the correlation between the two series was still good ($r = 0.89$), but the slope deviated somewhat from unity ($y = 0.60x + 1.17$).

Discussion and Conclusions

Use of a ^{11}C-labeled tracer is often preferred in PET studies for several practical reasons. The 20-min half-life is long enough to allow some transport of the compound between labeling and PET facilities without too much loss due to radioactive decay as is true for oxygen-15 ($t_{1/2}$ = 2 min). However, its half-life is short enough to allow sequential measurements on the same individual within a relatively short period of time, as is not true for fluorine-18 ($t_{1/2}$ = 110 min).

A new tracer, [^{11}C]-4-isopropylantipyrine, has therefore been synthesized and evaluated as a tracer for regional blood flow. Rapid, efficient labeling was easily achieved with $^{11}CH_3I$ in dimethyl sulfoxide with solid potasium hydroxide as a base. The ^{14}C-labeled compound, [^{14}C]-1, was used to determine its blood–brain partition coefficient as 0.62 ± 0.03 in rats.

Three experiments performed under normocapnic conditions suggest that, at normal flow conditions, 4-isopropylantipyrine yields highly comparable blood flow results to those obtained with IAP. Isopropylantipyrine does not seem to be more diffusion limited than IAP since the flow values measured during hypercapnia for 4-isopropylantipyrine were higher than the values measured with IAP. On the basis of these biological studies it is proposed that [^{11}C]-1 is an appropriate tracer for measuring regional CBF in vivo by positron emission tomography.

Experimental Section

Melting points were determined in open-end capillary tubes and are uncorrected. Thin-layer chromatography (TLC) was performed on precoated Merck 60 silica gel plates with scanning by an LB 2723 Berthold radioscanner. Gas chromatography analyses were carried out on a Varian 1400 instrument with flame detection (3% OV-17 on Gas-Chrom Q, 100/120 mesh). Preparative high-performance liquid chromatography (HPLC) separations were performed on an LDC Constametric I pump, Rheodyne injector (Type 7126 with a 1-mL loop), a μ-Bondapak C-18 column, an LDC Spectromonitor II, and a GM tube for radiation detection. ^{1}H NMR spectra were obtained on a JEOL FX90Q spectrometer. Analytical grade tetrahydrofuran (THF), lithium aluminum hydride ($LiAlH_4$), hydriodic acid (HI), dimethyl sulfoxide (Me_2SO), acetonitrile, and 1-phenyl-3-methyl-pyrazol-5-one (**3**) were all obtained from Merck. [^{14}C]Methyl iodide was obtained from the Radiochemical Centre, Amersham, England.

4-Isopropyl-3-methyl-1-phenylpyrazol-5-one (2). A mixture of compound **3** and acetone dissolved in methanol was hydrogenated over Raney nickel as described by Volk.[14] The resulting product **2** was recrystallized from methanol–water: mp 117–118 °C (lit.[14] mp 114–117 °C); ^{1}H NMR 1.13 (6 H, t, $-CH(CH_3)_2$), 2.18 (3 H, s, $-CH_3$), 2.5 (1 H, m, $-CH(CH_3)_2$), 3.1 (1 H, s, NH), 7–8 ppm (5 H, m, phenyl).

[^{14}C]-4-Isopropylantipyrine ([^{14}C]-1). A solution of **2** (0.4 mg) in 200 μL of acetonitrile was combined with [^{14}C]methyl iodide (50–200 μCi) dissolved in 300 μL of acetonitrile and loaded into a 500-μL HPLC injection loop. The injector valve was closed and the loop heated in an oil bath maintained at 150–170 °C for 15 min. The pressure generated by the expansion of solvent at this high temperature was maintained efficiently by the closed valve, and essentially no loss of solvent or tracer was observed in the labeling experiments. After cooling, the ^{14}C-labeled 1 was separated from starting material and other labeled byproducts by preparative radio–TLC: ethyl acetate–toluene = 1:1, R_f 0.4–0.45, cospotted with standard reference 1. With a radioscanner, product distribution was determined to be 1 (60–80%, R_f 0.4–0.45) and two byproducts eluting with R_f = 0.05 and 0.95 (total percent varying 20–40%). Since unreacted [^{14}C]methyl iodide was not detectable by the TLC system, samples of the solution before and after heating and of the isolated product were analyzed by scintillation counting. Incorporation of the [^{14}C]methyl iodide varied from 30 to 70%, resulting in a total yield of 10–20% for [^{14}C]-1 under these reaction conditions.

The desired product was isolated by stirring the silica gel overnight with ethanol, filtering, evaporating to dryness, and redissolving in ethanol to the desired concentration. The resulting solution was stored under nitrogen in the refrigerator. The [^{14}C]-4-isopropylantipyrine thus isolated was shown to migrate on TLC with the commercially available reference material and was likewise proven to be stable under the storage conditions. Specific activity, determined by comparison with a standard GC curve for the reference compound, was essentially the same as that of the [^{14}C]methyl iodide used in the labeling experiment.

Radiolabeling with ^{11}C (Figure 1). [^{11}C]Methyl Iodide. $^{11}CO_2$ was produced with a Scanditronix RNP 16 cyclotron by

(12) Abdul-Rahman, A.; Dahlgren, N.; Ingvar, M.; Rehncrona, S.; Siesjö, B. *Acta Physiol. Scand.* **1979**, *106*, 53.
(13) Kågström, E.; Smith, M.; Siesjö, B. *Acta Physiol. Scand.* **1983**, *118*, 281.
(14) Volk, H. Ger. Patent 962 254, April 1957; *Chem. Abstr.* **1959**, 10253a.

the irradiation of nitrogen gas by 16-MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction. After being trapped in a loop cooled by liquid nitrogen, the $^{11}CO_2$ was transferred by a stream of nitrogen into a cooled vessel (-78 °C) containing $LiAlH_4$ (10 μmol) in THF (500 μL). At the end of the trapping, the THF was removed under vacuum while heating to 130 °C. The vessel was once again cooled to -78 °C, and HI (1 mL, 54%) was added. Subsequent heating to 130 °C resulted in distillation of the $^{11}CH_3I$ formed. After passing through traps of soda lime and phosphorus pentoxide, the $^{11}CH_3I$ was trapped in 200 μL of Me_2SO. The amounts of activity obtained were dependent on the length of the irradiation of the target. A typical result was the preparation of 5.5 GBq (150 mCi) of [^{11}C]methyl iodide 7-8 min after end of bombardment (EOB) (30 μA, 10 min) with a specific activity of 11.1-18.5 GBq/mmol (300-500 Ci/mmol).

[^{11}C]-4-Isopropylantipyrine ([^{11}C]-1). The Me_2SO solution of [^{11}C]methyl iodide described above was transferred to a test tube containing **2** (1 mg, 0.0046 mmol), 100 μL of Me_2SO, and several grains of solid KOH. The mixture was stirred vigorously at 70 °C for 5 min. At the end of the reaction, 200 μL of the mobile phase for the HPLC was added and the resulting solution was injected onto the column (μ-Bondapak C-18, 300 × 7.8 mm, Waters). The mobile phase was a mixture of acetonitrile and H_3PO_4 (0.01 M) (35:65). A flow rate of 4 mL/min was used. The UV absorption at 241 nm and radioactivity were monitored simultaneously. The labeled [^{11}C]-1 eluted after 10.5 min, well-separated from labeled byproducts. The product was collected, evaporated to dryness in a rotary evaporator, and dissolved in a mixture of 3.5 mL of propylene glycol and 1.5 mL of ethanol. After addition of 5 mL of physiological saline and filtration through a Millipore filter (0.22 μm), a solution sterile and free from pyrogens was obtained: yield 40-50%; sp act. 3.7-7.4 GBq/mmol (100-200 Ci/mmol); time of preparation from EOB 40 min.

Animal Experiments. Male Wistar rats were used. The experiments were performed by the method described in Dahlgren et al.[15] for paralyzed and ventilated rats. Only a brief description of the main procedure will be given here. The animals were anesthetized in a jar using halothane (3.5%) in a mixture of oxygen and nitrous oxide (30:70). When unresponsive, they were tracheostomized and paralyzed (tubocurarine, iv 2 mg/kg), and then the halothane was decreased to 0.7% for the duration of the operative procedure which included placement of the necessary arterial and venous catheters. Blood pressure was over 100 mm Hg in all animals, P_{O_2} was adusted to surpass 100 mm Hg, P_{CO_2} was 36.9 ± 1.0 mmHg, and temperature was adjusted to close to 37 °C.

Details of the procedure used in the measurement of the partition coefficient are given in Abdul-Rahman et al.[12] In order to minimize the loss of the isotope during the experiment, the kidney blood vessels and ureters were ligated. Four animals received a bolus dose of [^{14}C]-1 (150 μCi/kg) at the start of the experiment and were then allowed an equilibration period of 45 min before decapitation. Blood samples taken during this period were frozen in liquid nitrogen. Radioactivity was then measured, in tissue and blood samples, using β-scintillation counting. The efficiency of the measurements was evaluated by adding internal standard to each sample ([^{14}C]hexadecane).

In the determination of CBF, [^{14}C]-1 was dissolved in Kreb's solution and infused at a steady rate over a period of 45 s (normocapnia) or 20 s (hypercapnia). During the infusion a set of timed blood samples were taken from the brachial artery, the last sample being taken at the time of decapitation. The brain was removed, frozen, and sectioned in 20-μm slices that were subsequently allowed to expose an X-ray film along with a set of calibrated standards for 1 week. The radioactivity was then assessed densitometrically by using an aperture of 1 mm (Macbeth TD 501). Calculation of the flow rates was performed by the method of Sakurada et al.[4]

In three animals CBF could not be calculated due to the precipitation of the tracer in the infusion solution. 4-Isopropylantipyrine is only slightly soluble in water. Since the specific activity of [^{14}C]-1 was so low (essentially the same at that of the [^{14}C]methyl iodide used in the labeling), dissolution was not complete in those cases in which the mass of the tracer used exceeded its solubility limit. This problem can be avoided in future studies by dissolution in a more lipophilic solution such as ethanol-propylene glycol-physiological saline (1.5:3.5:5). This solvent composition is often used for dissolution of lipophilic pharmaceuticals and is not known to affect cerebral blood flow in these low quantities.

Registry No. [^{14}C]-1, 96964-35-1; [^{11}C]-1, 96964-36-2; **2**, 50993-68-5; $^{14}CH_3I$, 16170-82-4; $^{11}CH_3I$, 54245-42-0; **3**, 89-25-8.

(15) Dahlgren, N.; Ingvar, M.; Siesjö, B. *J. Cerebral Blood Flow Metab.* **1981**, *1*, 429.

Quantitative Evaluation of the β_2-Adrenoceptor Affinity of Phenoxypropanolamines and Phenylethanolamines

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The influence of the aromatic moiety of β-adrenoceptor ligands on the affinity for the β_2-adrenoceptor has been studied. Three classes of ligands have been examined, viz. *N*-isopropyl- and N-*tert*-butylphenylethanolamines and *N*-isopropylphenoxypropanolamines. Computer-assisted analysis of the inhibition by any of these ligands of the specific (-)-[^{3}H]dihydroalprenolol binding to the β_2-adrenoceptors of a bovine skeletal muscle preparation in the presence of GppNHp (10^{-4} M) yielded the affinities of these ligands at pH 7.5. The obtained values were adjusted for the amounts of cations present at this pH value. A significant correlation was found between the calculated lipophilicities and the experimentally determined affinities in the three classes. Furthermore, steric factors seem to play an important role, as these correlations were improved by the introduction of steric parameters for the aromatic substituents in the regression analyses. From the established equations it is concluded that the phenoxypropanolamine derivatives bind to the β_2-adrenoceptor in a way different from that of the ligands in both ethanolamine classes.

The molecular basis of the interaction between the β-adrenoceptor and its ligands has been studied extensively.[1] The availability of radioactive ligands like [^{3}H]dihydroalprenolol (DHA), [^{125}I]hydroxybenzylpindolol (IHYP), and [^{125}I]cyanopindolol (ICYP) has been and still is an enormous impetus for the unraveling of triggering and regulatory steps in the hormone-receptor interaction. Surprisingly, qualitative considerations as well as quantitative analyses concerning the putative relation between physicochemical properties and biological activity (i.e., affinity and/or intrinsic activity) of β-adrenoceptor ligands

(1) A number of reviews have appeared, e.g.: Lefkowitz, R. J.; Caron, M. G.; Michel, T.; Stadel, J. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1982**, *41*, 2664. Also: Caron, M. G.; Shorr, R. G. L.; Lavin, T. N.; Lefkowitz, R. J. *Metabolism* **1982**, *31*, 658.

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