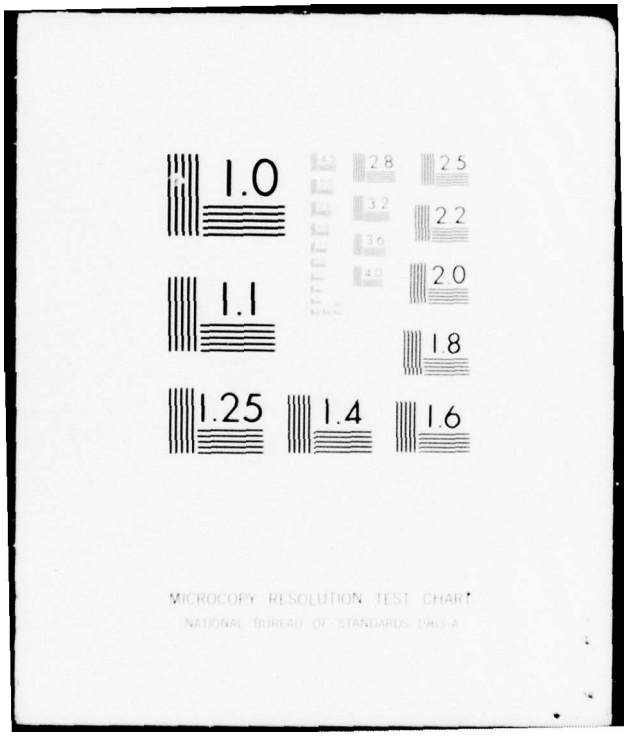
AD-A042 845 JOHNS HOPKINS UNIV BALTIMORE MD DEPT OF ENVIRONMENT--ETC F/G 6/1
BIOCHEMICAL ANALYSIS OF CEREBROSPINAL FLUID IN RESPONSE TO DRUG--ETC(U)
JAN 74 B BURNS DADA17-73-C-3129

UNCLASSIFIED NL

1 OF 1
AD
A042845

END
DATE FILMED
9-77
DDC


1.0
1.1
1.25
1.4
1.6
2.8
2.5
3.2
2.2
3.6
2.0
4.0
1.8
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963 A

UNCLASSIFIED

Biochemical Analysis of Cerebrospinal Fluid in Response to Drug Administration.

REPORT NUMBER 03

Annual Summary Report

Barry Burns, Ph. D.

7 Jan 74

41 p.

Rept. no. 3
(Annual) 1 Jun 73 - 31 May 74,

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Washington, D. C. 20314

Contract No. DADA 17-73-C3129

THE JOHNS HOPKINS UNIVERSITY
Baltimore, Maryland 21205

DDC AVAILABILITY STATEMENT

Approved for Public Release: Distribution Unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

401 924

SUMMARY

The general objective of this program was to evaluate the neurochemical and physiologic effects of some commonly abused drugs. We have completed the last stage of development on the chronic primate model by designing a new technique for exiting subcutaneous devices or catheters. The new procedure is maintenance free and has a zero incidence of infection in three animals. We have obtained some information on diurnal variations in CSF production rate and CSF levels of MHPG. Like many other investigators utilizing rhesus monkeys for biomedical research we were unable to obtain any large adult males during the first two quarters of this year. Monkeys have now arrived and we will utilize some of them for drug studies during the two quarters of the current year. We have used the absence of primates as an opportunity to conduct several series of acute experiments on dogs, and have developed a new model for the study of CSF neurochemistry and drug abuse. The bulk of the work discussed in this report derives from these experiments.

In the dog studies we found that d-amphetamine (3mg/kg) produces a two to threefold increase in CSF levels of the norepinephrine metabolite MHPG. L-amphetamine on the other hand does not significantly elevate the CSF MHPG levels.

Cerebral blood flow increased threefold with d-amphetamine and only slightly with the l-isomer. Arterial pressure doubled after d-amphetamine and increased 20-30% after the l-isomer at the same dose.

The d-amphetamine isomer also increased CSF production rates and decreased the Cl^- permeability of the blood-brain barrier. A close correlation was observed between CSF production and cerebral blood flow after d-amphetamine. There was an increase in total metabolic rate after d-amphetamine but not after l- at 3mg/kg.

The effect of the d-isomer on cerebral blood flow was shown to be due to the direct vasoactive nature of d-amphetamine, and CSF production rate increased secondarily to increased cerebral blood flow.

Initial studies of the canine peripheral circulation using a right-heart bypass preparation revealed that peripheral dopamine release increases cardiac output, blood pressure and mean systemic pressure.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Resources, National Academy of Sciences, National Research Council.

TABLE OF CONTENTS

List of Appendices, Illustrations and Tables

BODY OF REPORT

PROBLEM

Our goal was to evaluate the CNS neurochemical and cardiovascular circulatory response to certain commonly abused drugs. These studies involved both chronic and acute experiments on primates and dogs, respectively.

BACKGROUND

There are a number of methodological difficulties which arise during repeated sampling of CSF in chronic animals. Foremost among these is the significant dead space in most systems, compared with the volume of CSF which can be withdrawn discretely. It has also been observed that in-dwelling ventricular or sub-arachnoid catheters will fibrose in less than two or three weeks time (ommaya, personal communication).

Clearly, drug addiction and abuse are a function of several variables related to the biochemical nature of certain compounds and the nature of the total drug effect on the physiology of the species being studies. The basic biological effects of a drug are key to understanding preference, abuse and addiction. In the initial contract proposal we planned to study the sequence of neural-biochemical events associated with drug administration in unanesthetized primates, but emphasized that existing techniques for sampling CSF were inadequate and were inconsistent with methods for measuring behavioral and cardiovascular effects simultaneously.

We felt that if reliable samples of CSF could be obtained remotely from chronically prepared monkeys maintained in an isolated environment so that interactions with the experimenter would not interfere with the normal drug effects, we would be in a position to determine drug interaction with a number of different neurotransmitter systems simultaneously and conduct repeated experiments on the same animal to better compare the different effects. Although not specifically implicated in the original proposal, we have incorporated cardiovascular parameters in these drug studies because of their value in relationship to the central norepinephrine transmitter systems controlling blood pressure, heart rate and respiration. These

measures have proved valuable in evaluating drug action, and we would like to expand on these and continue this type of measurement in future research efforts.

EXPERIMENTAL APPROACH

Chronic Primate Studies:

CSF Collection. A detailed discussion relating to the surgical procedures and design of chronic devices for use with primates was presented in Report 02, 11 September, 1973, and will not be duplicated here. Insofar as the actual collection of CSF, we have settled on a design which allows the samples to be collected each hour and maintained at 0-10°C, ± 0.3°C. The fraction collector (Gilson FC80-E) is mounted on the rear of the primate booth in a small insulated box with transparent top and front sides for visually checking the system operation. The samples are centrifuged 5 min. at 3,000 RPM, and decanted into small storage vials. At present, samples are then stored in the refrigerator due to space limitations in the liquid nitrogen container. We have also experienced a significant sample loss from LN_2 storage during the sample transfer to dry ice, for transport to Walter Reed. For this reason we are requesting an adequate freezer in the renewal application. Temperature is critical for storage of parent catecholamines, and long term storage in general. Often there is several months lapse after sample collection before the assay is conducted.

The refrigeration system for the fraction collector is mounted on the lower rear portion of the booth, with flexible cables leading to the refrigeration coil in the fraction collector housing.

The refrigerated fraction collector sits on a vertical track which can be set for any desired height with respect to the monkey. The tubing leading from the monkeys chronic head column to the needle mounted in the collection device is sterile and is replaced every 3-4 days using aeseptic techniques.

Acute Dog Studies:

The Unanesthetized Preparation. For the interim period during which primates were not available, we have conducted many of the experiments originally designed for monkeys on dogs. In order to have meaningful data which we could relate to the primate work, it was necessary for us to develop a preparation in which general anesthetics were not used. All catheters are placed percutaneously and the skin is first infiltrated with a long-acting local anesthetic.

(Pontocaine). The procedure is as follows: the dog is given 20 mg Succinyl choline intravenously, and within 10 sec. is motionless. The larynx is then sprayed with a rapid, local anesthetic (Cetacaine, Cetylite Industries, Inc.), the trachea intubated and the animal ventilated artificially with a Harvard respirator. The entire procedure takes less than 30 sec and the dog is at no time subject to any physical discomfort. The eyelids are taped shut to prevent visual stimulation and drying of the corneal membranes. The next step is to record a bipolar ECG from chest electrodes. Heart rate is monitored from the ECG at present. Heart rate and blood pressure are the primary indices of stress to the animal, since any general excitement or peripheral release of catecholamines affects these two parameters immediately (Fig. 6A,B).

Control of Respiration. In all experiments involving muscle relaxants, the dog is ventilated at a fixed volume on a Harvard respirator. End-tidal PCO_2 is continuously monitored with a Beckman LB-1 infrared capnograph, and is maintained at 5-6%, depending on the blood-gas readings which are taken periodically to ensure adequate oxygenation and normal arterial PCO_2 levels. Blood pH is maintained at 7.3 - 7.4 by adjusting the respiratory rate. A continuous infusion of Succinyl choline is maintained in lactated ringers solution through a 16 ga. intracath placed in the antebrachial vein percutaneously. The infusion rate for Succinylcholine is 0.96 mg/min at a concentration of 0.8 mg/ml in the infusion fluid.

Blood Pressure Measurement. Catheters are inserted into the femoral artery and the antebrachial vein percutaneously. The skin is first deadened locally with Pontocaine infiltration (10 mg/ml). In other experiments to be described later, in which general anesthesia was used, a cut-down was made on the femoral artery and vein for the placement of catheters.

For measurement of left-ventricular pressure or right atrial pressure, catheters were advanced from the femoral region while looking for characteristic waveforms. Drug infusions were made through the 16 ga Intracath placed in the antebrachial vein.

CSF Collection. The skin and epi-dural region are infiltrated with Pontocaine (Winthrop Lab.), and a 16 ga Intracath (Deseret Pharm.) is placed in the cisterna magna through the atlanto-occipital membrane. Once the catheter is in place, the end is lowered some 4-10 cm with respect to the cranial midline in the prone position, and the CSF is collected gravimetrically at a constant outflow pressure.

This method of CSF collection allows us to estimate the CSF production rate volumetrically, and this figure is used in the calculations relating the drug response to actual CNS output of neurotransmitter per-unit time (Fig. 3).

Experimental Protocol. After catheter placement, control samples of CSF are collected for 2 hours pre-drug, and up to 4 hours post-drug. All physiologic parameters are recorded continuously on a Brush

8-channel recorder for subsequent data reduction by hand. Short representative sections of experiments are recorded on FM magnetic tape for permanent records, however we are only able to record 6 channels of data on tape.

Hourly blood samples are taken and the plasma frozen and stored for future analyses. CSF is taken in half-hour samples, centrifuged 1 min at 12,000 rpm, and frozen and stored in acid-washed vials.

Blood hematocrit is checked with every blood sample, and fluids administered I.V. to maintain the control level. The infusion rate for succinylcholine (roughly 2 mg/kg/hr) was determined empirically as the level which was just sufficient to prevent recovery of motor control during the experiment. It is metabolized by a non-specific plasma pseudocholine esterase.

Estimation of Metabolic Rate. By artifically ventilating the dog at a fixed rate, any variations in end-tidal PCO_2 which reflect steady-state changes serve as qualitative and quantitative indices of changes in metabolic rate. As will be discussed later, we have observed that d-amphetamine does increase the metabolic rate, and this may be somehow related to its effects on cerebral blood flow and CSF secretion rates (Fig. 13).

Acute Dog Studies using General Anesthesia:

The Cerebral Blood-Flow Preparation. Our original intent was to relate the drug effects to cerebral blood flow in the monkeys; and we felt that this information was equally important in the dog studies which were designed to complement the primate work. A slightly different technique must be used in the dog however, since the intracranial circulation has a large number of anastomoses with the extracranial vascular bed. This precludes simple placement of a flow probe on the internal carotid, as can be done in primates. Instead, a preparation referred to as a 'torcular-venous shunt' is used in the dog. The procedure is fairly simple, and involves isolating the venous outflow from the cerebral vascular bed, and passing this outflow through an extracorporeal flow probe. Blood is then returned to the circulation by an external pump whose speed is regulated by the rate of blood flow (Fig. 12). The method was originally devised by Rapela and Green (1964), and we thank R. Traystman of this department for his assistance in these studies. Na pentobarbital (35 mg/kg) was used as the anesthetic agent in the brain blood flow studies. Although we do not know the extent to which pentobarbital interacts with amphetamines, this will be the topic of future experiments. The preparation is generally very responsive to drugs, and we suspect that anesthesia is not critical.

The Ventriculo-Cisternal Perfusion. We have undertaken perfusions of the ventricular system for two reasons: first, although the volumetric method of estimating CSF production rates is approximately correct, there are other factors such as blood pressure and cerebral intracranial venous compliance which can affect the amount of CSF leaving the system at a constant outflow pressure; secondly, this preparation is necessary in order to accurately measure the transfer of substances from CSF to blood, or vice-versa, i.e., the permeability of the blood-CSF barrier. The main reason for attempting these permeability studies is that we are interested in the extent to which drug effects are direct actions on neurons or other secondary actions on permeability, oxygenation, etc. (the type of things which clearly can affect neuronal excitability and cerebral function through indirect ways). If we are to understand the drug response, we must be able to rule out these secondary physiologic changes or else be able to incorporate them somehow into the drug action. From a more practical standpoint, we have already indicated that the CSF production rate is very important in the interpretation of a drug effect, since the pharmacologist can only tell us the concentration of a substance in the CSF per unit volume - and no matter what the drug effect is, it can be negated, reversed or accentuated by the simple expedient of changing the rate at which the CSF is being produced and leaving the system.

The blood-brain barrier permeability is also important for other reasons: For obvious reasons the concentration of substances in the neuronal extracellular fluid (and subsequently CSF) is rigidly controlled. Many catecholamines are present in blood plasma at levels far in excess of the CSF levels, and the ability of the blood-brain barrier to exclude these compounds may well change after the administration of drugs which have pronounced peripheral effects on the circulation. It is also clear that if the dependent variable in a study is the CSF level of a substance (MHPG, for example) then we must be able to establish to what extent changes in CSF levels are due to parenchymal sources and to what extent these changes may be due to similar compounds leaking into the CSF from the blood. The experiments we plan to conduct with the ventriculo-cisternal perfusion technique in the future will be designed to answer these important questions.

In the perfusion experiments, we have used anesthetized animals (Na pentobarbital, 35 mg/kg priming and 10 mg/kg/hr sustaining). An outflow catheter is first inserted into the cisterna-magna, as described under "CSF Collection" previously. Following this, a small hole is drilled in the skull approx. 6 mm from the midline, perpendicular to the plane of the top of the mid-sagittal crest, and midway between the lateral canthus of the eye and the external occipital protuberance. A 16 ga needle with a fluid-filled length of tubing attached is then stereotaxically lowered into the brain vertically until the ventricle is penetrated and fluid enters the ventricle due to the hydrostatic pressure of the fluid column (approx. 15 mm from the dura).

The ventricular system is then perfused with artificial CSF equilibrated with 5% CO_2. The composition of this fluid is as follows:

NaCl	7.4 g/l
KCl	0.22 g/l
$NaH_2PO_4 \cdot H_2O$	0.066 g/l
$NaHCO_3$	2.2 g/l
$CaCl_2$	0.2 g/l
$MgSO_4 \cdot 7H_2O$	0.1 g/l
Urea	0.4 g/l
Glucose	0.65 g/l

This mixture is nearly identical to normal dog (and monkey) CSF. The solution is autoclaved prior to the addition of Urea and Glucose, and then filtered through a 0.22 micron millipore filter prior to freezing and storage in sterile, acid-clean glass containers. Each bottle is also cultured prior to freezing and storage. Ideally, the solution should be adjusted to the osmolarity of each dogs CSF before each experiment, but we do not have access to an osmometer at present. The urea is used to regulate osmolarity.

The outflow catheter from the cisterna magna leads to a Gilson fraction collector (FC80-E), which changes the sample every 5 min. The flow rate through the system is varied between 50-500 microliters per-min. At the conclusion of each experiment, Trypan blue dye is added to the perfusion fluid, and the perfusion continued for an additional 15 min. At this time the dog is terminated, and the brain removed to verify the perfusion location and inflow needle placement. The areas in contact with the perfusion fluid are stained by the dye, whereas other areas remain colorless (Fig. 14).

The dogs are artificially ventilated to maintain the blood pH and PCO_2 within normal limits. Periodic blood samples are taken for estimation of PO_2 and %saturation.

Calculation of CSF Secretion Rate.

The dilution of a labeled, non-diffusible indicator is used to estimate the CSF production rate. For this purpose, we add ^{14}C-Inulin to the perfusate. Samples of the inflow and outflow CSF are taken and prepared for liquid scintillation counting, using a Packard Tri-Carb counter. In the absence of any CSF production, the outflow radioactivity (C_o) should equal the inflowing radioactivity (C_i), since the inulin molecule is too large to enter the blood or leave the CSF compartment. Some movement into the tissue usually takes place, but this is usually at a steady state, or complete, by 25 min after the beginning of perfusion. With the production of CSF, the inulin is essentially diluted as it passes through the system, and the extent of this dilution determines the CSF production rate. The inflow rate is controlled by an infusion pump, and is measured in μl/min. The system must obey the laws of mass action, therefore the flow in multiplied by the counts in must equal the total flow out, times counts out.

The relationship is expressed mathematically as follows:

$$\dot{Q}_{in}(C_i) = \dot{Q}_{out}(C_o) \qquad (1)$$

where: Q_{in} = flow in
Q_{out} = flow out

However, the term for flow out (Q_{out}) is in reality composed of two terms - the flow in, plus the flow due to CSF production. This means that $Q_{out} = Q_{in} + Q_{csf}$, and eq. (1) now becomes:

$$\dot{Q}_{in}(C_i) = C_o(\dot{Q}_{in} + \dot{Q}_{csf}) \qquad (2)$$

and upon solving for CSF production, we get:

$$\dot{Q}_{csf} = \frac{C_i - C_o}{C_o}(\dot{Q}_{in}) \qquad (3)$$

We find that this figure for CSF production rate agrees very closely with that given volumetrically on the same sample, in the steady state. The dilution method is less subject to error from transient changes in blood pressure, which may tend to change the volume of the ventricular system being perfused. This is because the production is based on the radioactivity per/ml of CSF on the outflow, and a sudden change in ventricular volume would not change the concentration/ml of the labeled non-diffusible indicator, it would only change the total volume collected for that time period. Both methods would be in error during transient changes however, if the CSF volume added originated from the subarachnoid compartment which was not being perfused. This error would only affect transient changes, and the steady-state measurements in either instance would be valid.

Permeability of the Blood-Brain Barrier. The ventriculo-cisternal perfusion system is ideally suited for permeability measurements. In order to estimate the permeability it is necessary to have some measure of CSF production, or dilution, in addition to comparing the net gain or loss of the substance of interest in the perfusion fluid. Permeability is defined here as the simple ratio of flux divided by the mean concentration gradient in the ventricular system.

In order to calculate the flux of the molecular species being transported into the blood by diffusion across the membranes comprising the blood-brain barrier, we must assume that in the absence of any transport, i.e., zero permeability, the compound of interest would be diluted to the same extent as the non-diffusible indicator (inulin). This means that the fractional recovery for inulin should be the same as that for chloride in the absence of chloride transfer to blood.

for ^{14}C-Inulin: $\frac{C_o}{C_i} = \%$ $= (F)$

If we add labeled chloride to the perfusion fluid ($^{36}Cl^-$), we can then predict the theoretical recovery of chloride in the outflow fluid - based on the inulin dilution - that would exist with no transfer.

$$F_{inulin}(^{36}Cl_{inflow}) = \text{Predicted } ^{36}Cl_{outflow}$$

The difference between the predicted chloride concentration in the outflow and the actual measured concentration of chloride in the outflow is equal to the amount of chloride lost to the system, i.e., flux. For each sample, this measure of flux is divided by the numerical average of actual in and out concentrations to obtain the permeability:

$$P_{chloride} = \frac{(F(C_i) - C_o)\dot{Q}_T}{\frac{C_i + C_o}{e}} \quad ; e = 2.713 \qquad (4)$$

where: F = fractional recovery for inulin
C_i = inflow counts for chloride
C_o = outflow counts for chloride
P = chloride permeability
$\dot{Q}_T$ = Total Flow

Standard double label counting techniques are used for the two labeled compounds. This same procedure would be used to study the permeability of any compound of interest.

Peripheral Circulatory Studies:

Right-Heart Bypass Preparation. We have observed that some drugs such as the amphetamines often have profound circulatory effects. What we do not know is whether these effects are mediated by a CNS reflex, in which case we would have a better idea of brain nuclei involved; or whether the effects are entirely peripheral in nature, resulting from direct stimulation of the vasculature or the release of catecholamines peripherally. It is important, and appropriate to examine these effects in greater detail. Studies of this type cannot be done on chronic animals, and the principal investigator has had considerable experience in acute studies on dogs of the type to be described. Although we have not previously asked for funding for dog studies, we now feel that their incorporation is essential to the proposed work.

These studies have basically two aims: First, to separate the peripheral and central circulatory effects; secondly, to identify specific neural transmitters involved in the peripheral response by the use of appropriate blocking drugs and possibly plasma assays for catechols. Since it has been shown by other investigators that the CSF levels of catecholamines are related to the plasma levels, and further that the plasma levels are severalfold higher than those in CSF, we feel that these circulatory/biochemical studies in conjunction with the permeability studies described above will provide useful new information on the relationships between central and peripheral drug effects.

In the bypass preparation, right atrial pressure is kept constant at zero by adjusting the height of a Starling resistor (Fig. 16) in the extracorporeal circuit. Utilizing this system, the mechanical pump will direct 100% of the venous return (or cardiac output) into the pulmonary artery, and thereby eliminate any effects on flow which may be due to the pumping ability of the right heart.

Healthy mongrel dogs weighing approximately 20 kg were prepared for surgery with Na pentobarbital 35 mg/kg priming, and 5 mg/kg/hr sustaining. Animals were ventilated with a Harvard respirator and the chest opened by a midline incision and thoracotomy. Heparin sodium was used as the anticoagulant at 5 mg/kg followed by 10 mg/hr. Blood pressure was monitored by catheters in the femoral artery, pulmonary artery and right atrium. Periodic samples were taken for blood-gas analysis. Values for mean systemic pressure (MSP) were obtained by stopping the bypass pump and allowing the pressures between venous and arterial circulations to equalize. The MSP is determined only by the blood volume and the distensibility of the vascular tree as a whole. The stop-flow values for MSP were obtained within 7-10 sec during which time Pressure in the right atrium plateaued. Venous return was measured between the mechanical pump and the right atrium by a Carolina Medical flowmeter, linear over the range 0-4 l/min. Calibrations were obtained at the end of the experiment using blood from the dog. In order to avoid large changes in hematocrit of the dog as complete bypass was effected, sufficient blood was removed from a donar animal prior to each experiment to fill the pump circuitry.

We have completed three of these experiments to date. In all of these experiments we infused dopamine HCl (Intropin), 0.1 mg/kg/min intravenously. Complete sets of control measurements were made prior to the drug infusion.

RESULTS

Acute Dog Studies:

Introductory Note. Many more studies have been conducted than will be reported on at this time. One reason for this is that we do not yet have all the biochemical analytical results for the remaining experiments, and will present the remainder of the data in the final report. A listing of experiments completed appears in the section of this report.

Effects of d-Amphetamine. In the unanesthetized dog preparation discussed previously, we have administered the d-isomer at 3 mg/kg, i.v. In many ways the results of these experiments are similar to primate experiments in which the dosage was only 1.5 mg/kg. Figure 1 depicts the changes in CSF production, estimated gravimetrically, for three typical experiments: one with d-amphetamine, one with l-amphetamine, and one control. In all instances, the d-isomer resulted in considerably greater increases in CSF secretion rates than the l-isomer.

Blood pressure effects of the d-amphetamine at 3 mg/kg, a level which is known to produce stereotypy in dogs, were nearly identical to observed changes in CSF production rates (Fig. 2).

Preliminary analysis results indicate that d-amphetamine at 3 mg/kg produces a three to fourfold increase in the CSF levels of the norepinephrine metabolite MHPG (Fig. 3), when corrected to equal output of MHPG/unit time. This figure points up the value of having the simultaneous estimate of CSF production in the interpretation of the results.

A summary of the results from three experiments are presented in Fig. 7. The increases in MHPG in these experiments were greater than any changes observed in other metabolites in the chronic primate experiments during the previous contract period.

We did observe many similarities with the primate work however, and you will note in Fig. 4A,B that the pressor and heart rate effects of the d-isomer are accompanied by cardiac arrythmias and PVC's. The measurement of an ECG has been a valuable addition because it has enabled us to clearly distinguish between the peripheral effects of the d- and l-amphetamine isomers. A detailed example of the ability of d-amphetamine to produce cardiac arythmia is shown in Fig. 4B. Compare Fig. 4 with Fig. 5, which is the l-isomer, and note the near absence of myocardial irritation at the same dosage level with l-amphetamine. Also note the diminished pressor effects with the l-isomer. In some of the experiments, the l-isomer did elevate blood pressure more than in the example given; and we suspect that there are some unknown factors involved in the response of an individual animal to the various isomers.

Effects of l-Amphetamine. In general we noticed that this isomer at 3 mg/kg exhibited some qualitative similarities to the d-isomer, but was much less effective in every single experiment with the unanesthetized dogs.

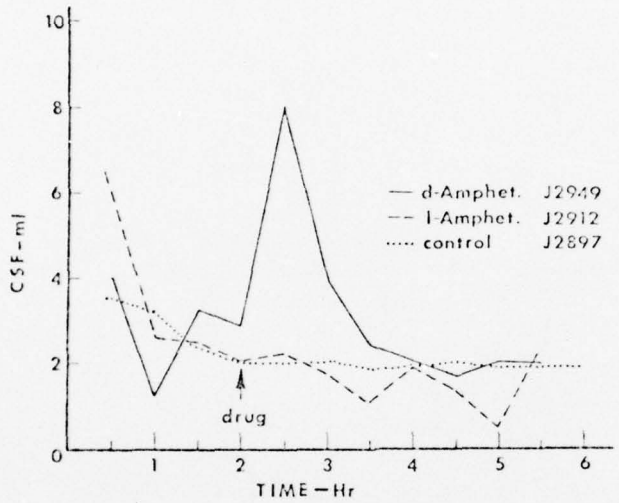


Figure 1. Differential effects of d- and l-amphetamine (3mg/kg,i.v.) on CSF Production rates in unanesthetized dogs. CSF was collected gravimetrically (at a pressure of -4 cm H_2O) with the dog in the prone position on its side. The data are plotted as mean values for each hour collection interval. The initial high values at T = 30 min., i.e., the mean for the first hour, is due to some initial drainage of CSF after the system is opened and approaches a hydrostatic equilibrium. In a stable animal, the CSF secretion rate is nearly invariant, and is in agreement with production rates in the literature obtained by different measurement techniques. The change after l-amphetamine, although minimal in dog J-2912, was clearly proportional to variations in blood pressure, and presumably cerebral blood flow.

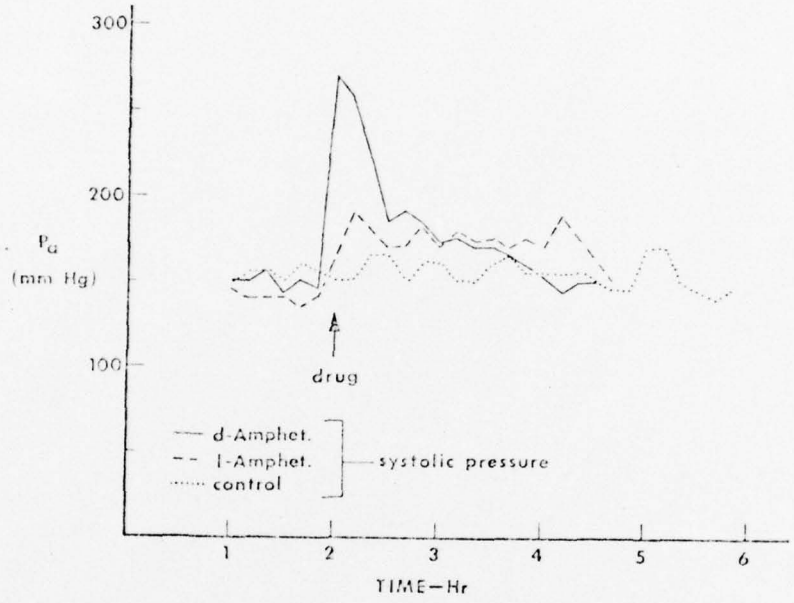


Figure 2. Systolic blood pressure changes induced by d- and l-amphetamine (3mg/kg, i.v.) in unanesthetized dogs. Values are plotted at 10 minute intervals for the same three dogs shown in Figure 1. Note the close relationship between arterial pressure and CSF production rates. Also note the decreased pressure response after the l-isomer at the same dosage level as d-amphetamine. Diastolic pressure changes (not shown) were similar to the systolic changes, with some increase in systolic pulse height after the drug.

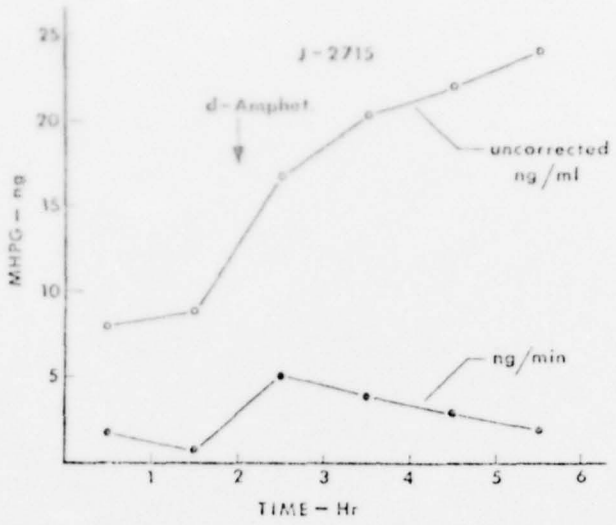


Figure 3. CSF levels of MHPG after 3 mg/kg d-amphetamine in the dog: Effects of correcting for CSF production rates. The CSF samples were pooled into hourly intervals, and data points represent mean levels for that hour. Both curves are from the same animal, and serve to demonstrate that as the CSF secretion rate varies, there is a subsequent concentration or dilution of MHPG. Changes in secretion rate for CSF (not shown) were similar to that shown in Figure 1 for d-amphetamine. The correction (ng/ml x ml/min CSF), although only approximate and confounded to some extent by blood pressure variations, still serves to demonstrate that there is an effective increase in brain output of MHPG nearly tripling after d-amphetamine. The corrected curve is also valuable because it relates the CNS effects very nicely to the variations in CSF production (cerebral blood flow) and arterial pressure changes after the drug; whereas this relationship would not be apparent using the upper curve of this figure which is uncorrected and simply reflects gross concentration per ml of CSF. Succinyl choline; unanesthetized.

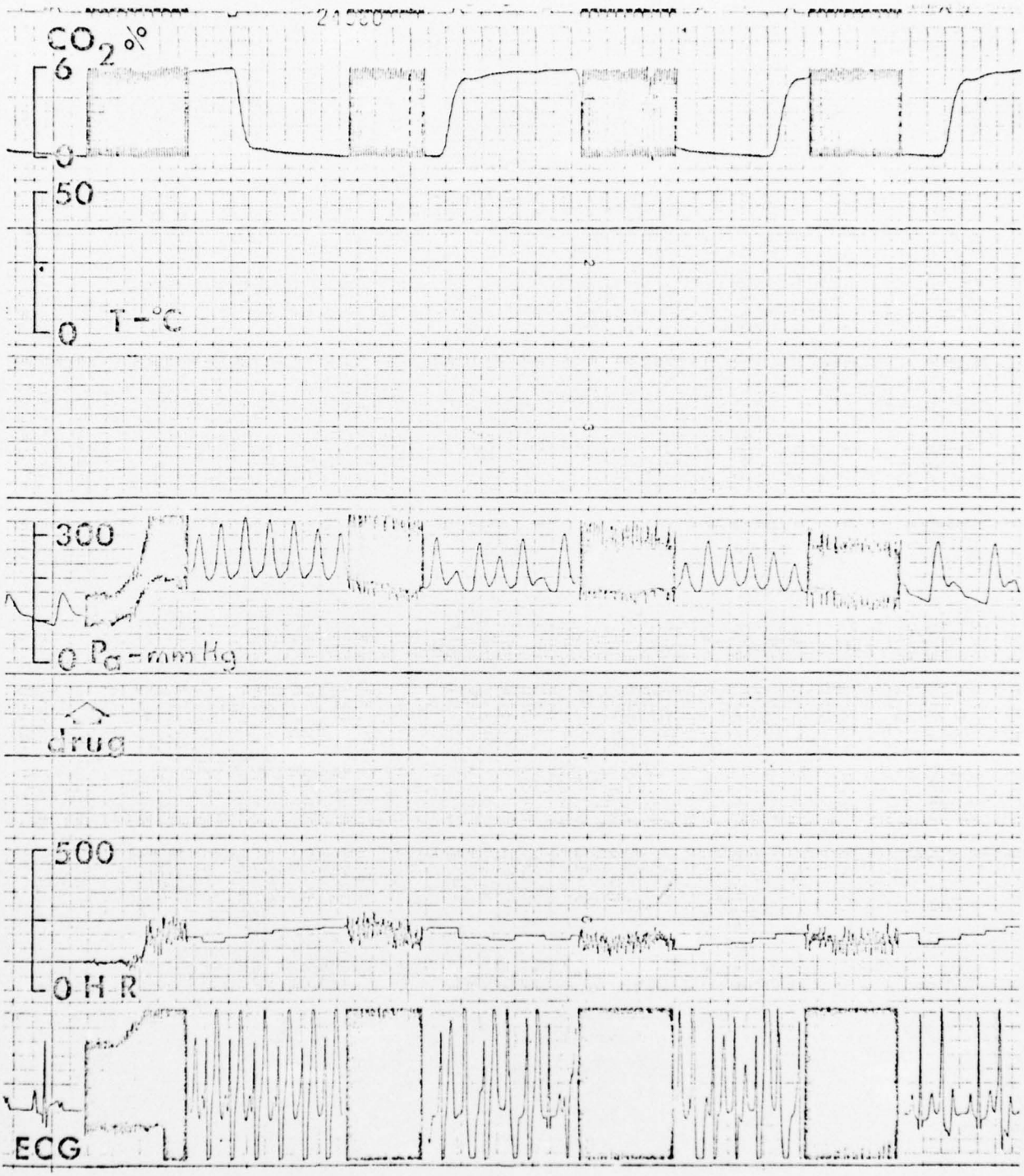


Figure 4A. Chart record for d-amphetamine. From the top down, the traces are end-tidal CO_2, rectal temperature, arterial pressure, heart rate and ECG (bipolar). Note the pronounced cardiovascular effects for the d-isomer at 3 mg/kg, i.v. Substantial heart rate increases are accompanied by left ventricular arrythmias and PVC's. Time scale is at top of record: downward marks are 10 sec. intervals; upward marks are 1 sec. intervals. Succinyl choline; unanesthetized.

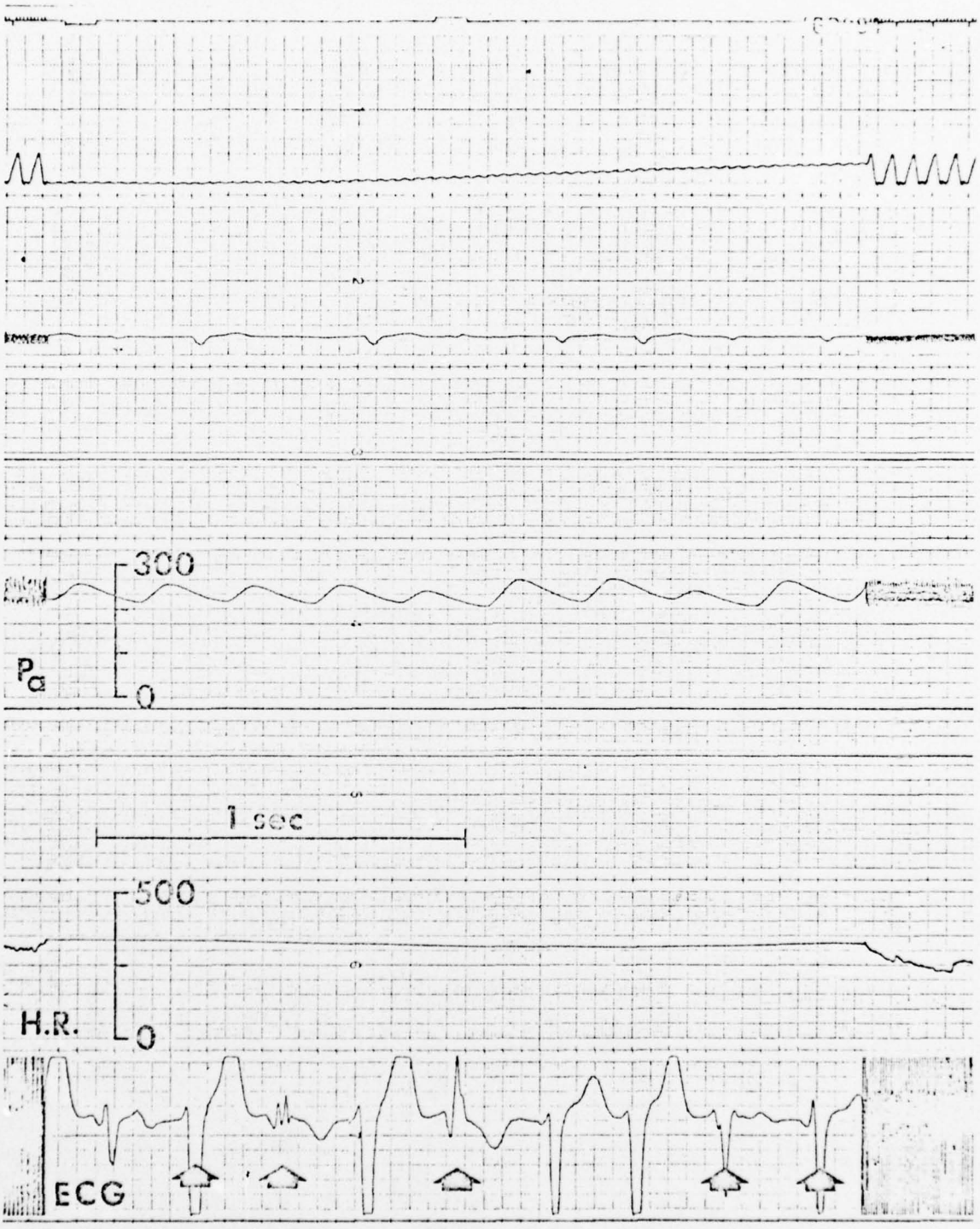


Figure 4B. Detailed example of myocardial effects for d-amphetamine (3 mg/kg, i.v.) in the unanesthetized dog. Note that five separate ventricular foci are apparent, resulting in a multi-focal ventricular rhythm characteristic of the d-amphetamine response. Foci = arrows.

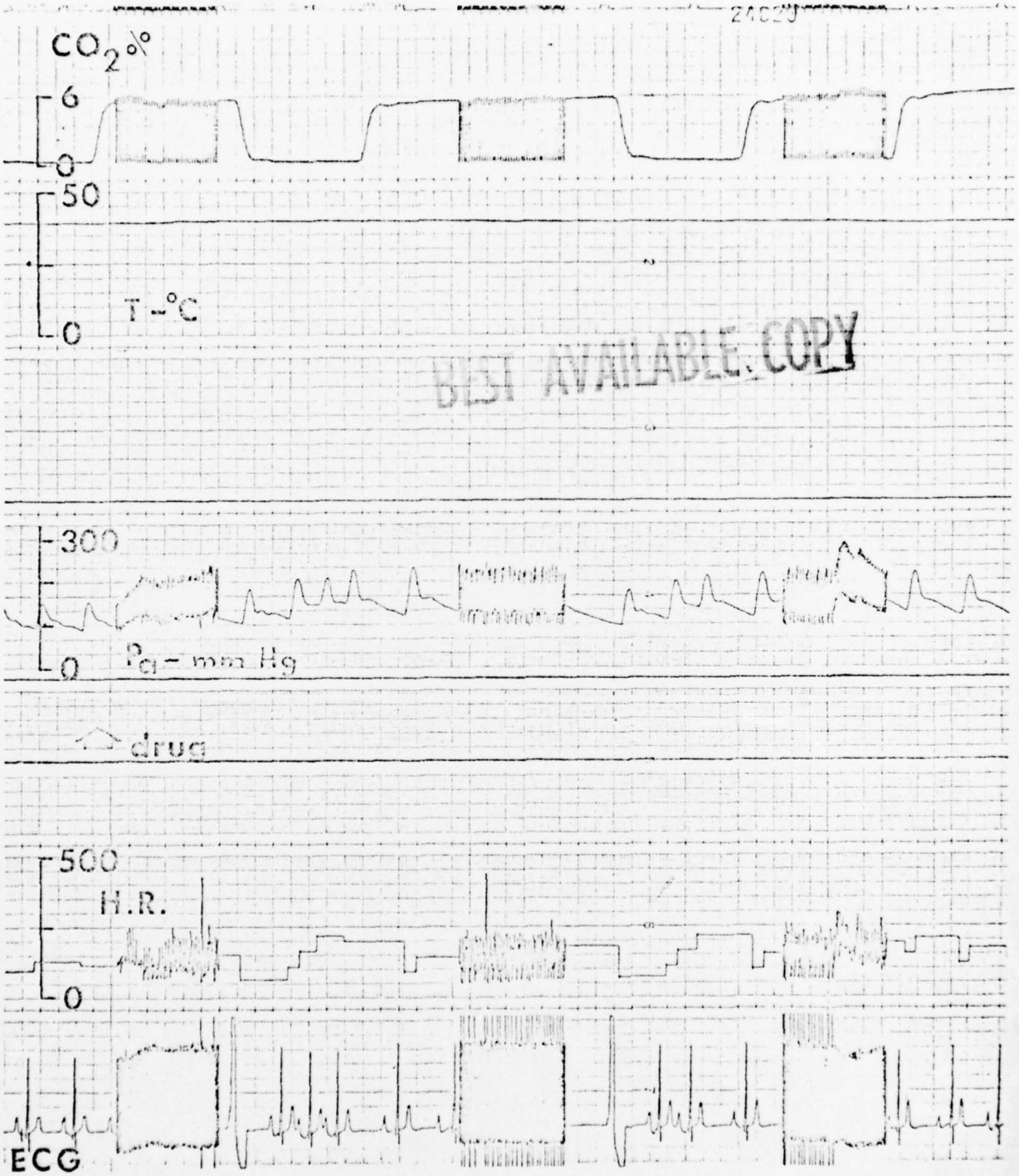


Figure 5. Cardiovascular response to l-amphetamine (3 mg/kg, i.v.) in the unanesthetized dog. Compare with fig. 4A, and note the diminished pressor response, diminished heart rate effects (primarily arrythmia), and near absence of ECG effects - only an occasional ventricular focus. Time scale as in fig. 4A.

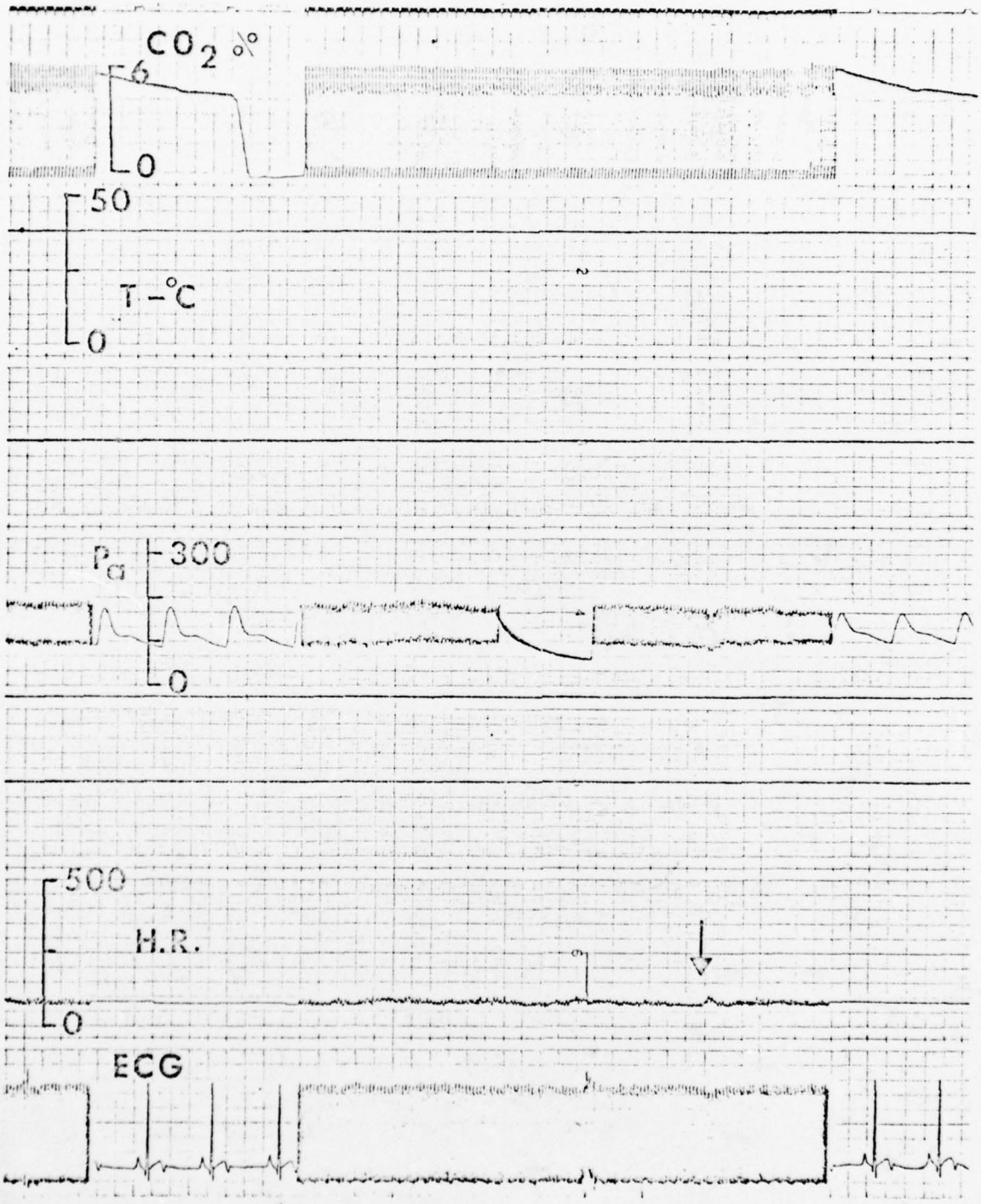


Figure 6A. The acute preparation. This record depicts the sensitivity of heart rate and blood pressure as stress indicators in this preparation, while also indicating the very stable nature of the dogs condition when the appropriate precautions are taken to eliminate any pain or discomfort to the animal. The tail was lightly pinched at the arrow on the heart rate channel. The sharp spike in heart rate preceding this is electrical artifact from flushing the arterial catheter. Note that flushing the catheter is unnoticed by dog.

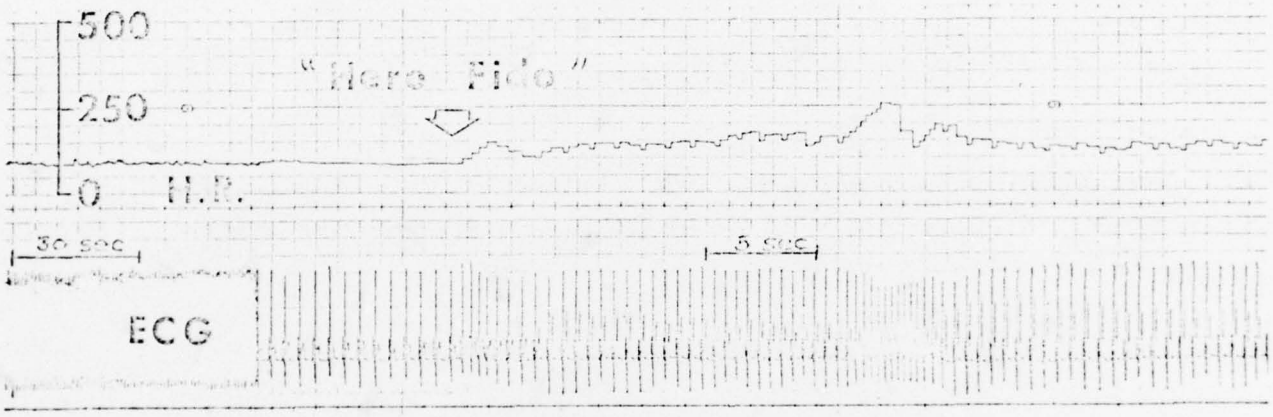


Figure 6B. The acute dog preparation.
This record demonstrates the alertness and responsiveness of the dog during a control period prior to the drug administration. At the point denoted by the arrow the dog was called in a moderate voice. Note the extremely stable heart rate prior to this at much slower recorder speed. Lower channel is bipolar ECG.

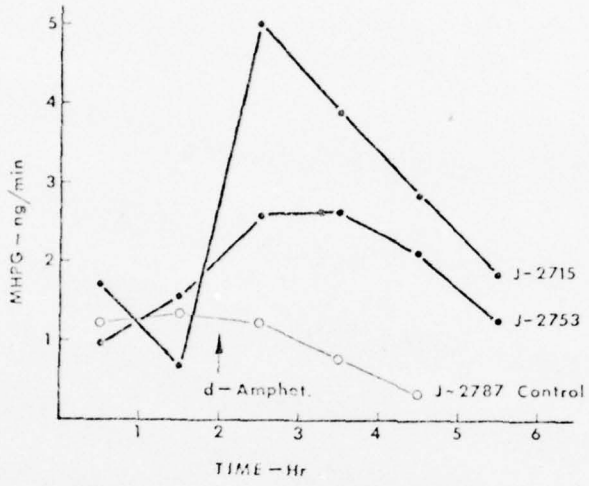


Figure 7. CSF levels of MHPG after d-amphetamine (3mg/kg i.v.) in two experimental and one control animal. These data are corrected for volume changes in CSF production rates, and expressed as the total brain MHPG output/min.

CSF levels return to near-baseline values within 4 hours after the drug. We observed parallel recoveries in CSF production rates, cerebral blood flow and systemic arterial pressure in similar experiments. Succinyl choline; unanesthetized.

Chronic Primate Studies:

Diurnal Studies of CSF Production. We have completed a series of chronic primate studies, using our previously developed model, in which we have followed the production of CSF over 24 hour periods. From these data we have been able to determine that there is a general trend in the production of CSF, which seems to follow the daily activity patterns to some extent. The possibility exists that these patterns reflect changes in brain blood flow and neuronal activity. This conclusion is based on the chronic studies in dogs in which these variables were well correlated, and will be pursued further in the discussion section of this report.

Figures 8 - 10 provide summary information of the diurnal production rates for CSF. There seems to be marked differences between the individual monkeys in terms of the absolute values (ml/hr).

The samples from one 24 hr period were analyzed for MHPG, and these data are shown in Fig. 11. We did not catheterize these animals because at the time the experiments were conducted, we were uncertain regarding the future availability of monkeys for this work. Cardiovascular data will be obtained in future experiments.

Anesthetized Dog Studies:

Cerebral Blood Flow. We found that d-amphetamine is extremely effective at increasing cerebral blood flow (Fig. 12, 13). At the same dosages used in the other experiments, there is an initial three-fold increase in cerebral blood flow (CBF). Although d-amphetamine also increases the blood pressure tremendously, we were able to show that the effect on CBF is independent of pressure and is somehow related to the basic mechanism of action of amphetamine on the CNS. Two possible mechanisms of action will be presented in the discussion section of this report.

Correlated with the amphetamine increase in CBF, there is an increase in total metabolic rate, and presumably CNS metabolic rate as well - which we will examine in future experiments. The increase in CBF and metabolic rate is much less with the l- than the d-isomer. We observed one experiment with no change in CBF after 3 mg/kg of l-amphet. We will be conducting similar experiments on primates in the future.

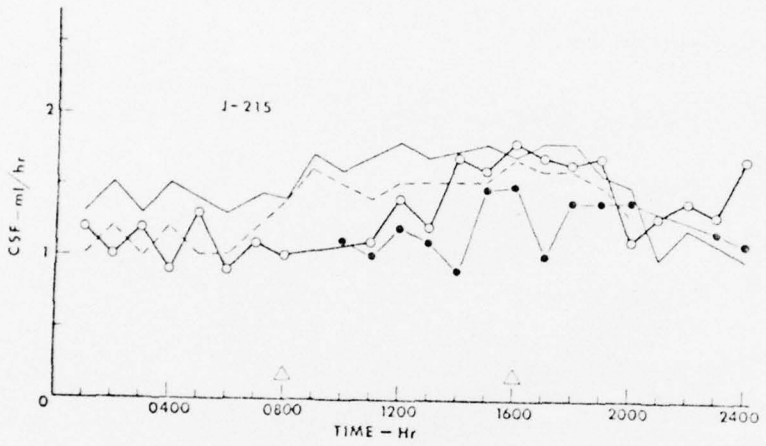


Figure 8. Diurnal CSF production variations in primate J-215. CSF was collected hourly at constant outflow pressure (-3cm H_2O with respect to the interauditory zero plane) in the refrigerated fraction collector. There is a trend to increased volumes during the day, especially in the evening, at which times activity is also increased. Small triangles indicate the time at which the monkey is fed. At all other times the booth door is closed and the monkey undisturbed. Some of the time from 0800-1400 hours is usually spent lightly dozing, with activity (not shown) increasing in the evening until 1900 hours.

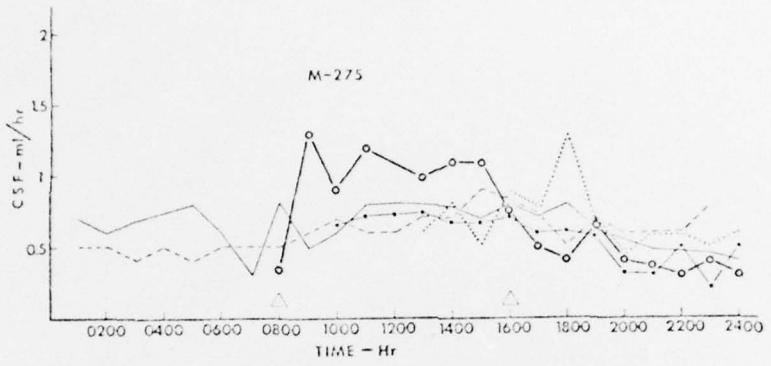


Figure 9. Diurnal CSF production variations in primate M-275. A similar trend to that in J-215 is seen in this monkey. Triangles represent feeding; at all other times the booth door is closed. On some days a complete 24 hour sample was not obtained due to catheter blockage. Whenever this occurred, the catheter was flushed with sterile ringers solution and the collection started again.

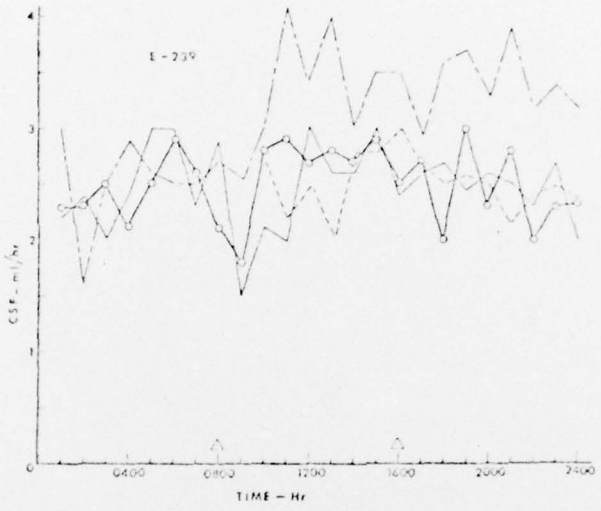


Figure 10. Diurnal CSF production variations in primate E-239. The trend to a distinct diurnal variation was most apparent in a single 24 hour period, in which the greatest volumes of CSF were also collected. We suspect that this indicates lack of any temporary blockage or catheter compression for this period, so that representation of true diurnal fluctuations would be most pronounced for this one day. Similar trends however can be seen in the other 24 hour periods but they are not so distinct. Collection techniques as in Figure 8 and 9. Triangles represent feeding.

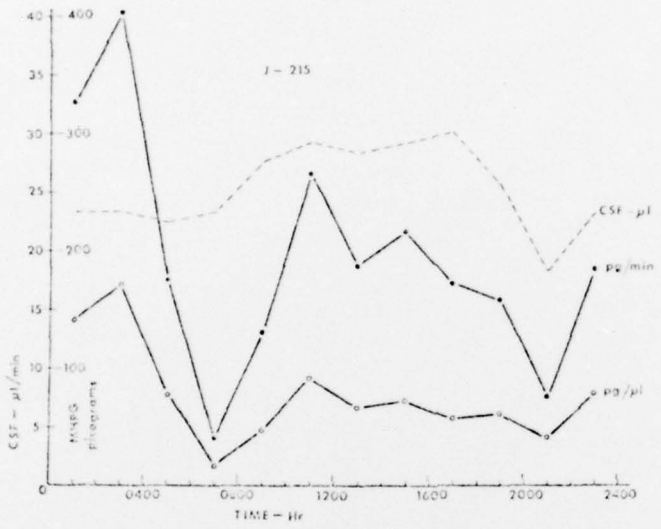


Figure 11. Diurnal variations in CSF MHPG levels in primate J-215. Here we have plotted the simultaneous CSF production rates and MHPG levels in the same CSF sample. For comparative purposes we have shown the uncorrected and corrected MHPG levels. In this instance the correction for CSF volume produced tends to magnify the diurnal variations in central MHPG output. There is an observable positive correlation here between the CSF secretion rates and MHPG levels. The hourly samples were pooled into two-hour intervals prior to analysis for MHPG, and the data are plotted at the mid-point of each two-hour interval.

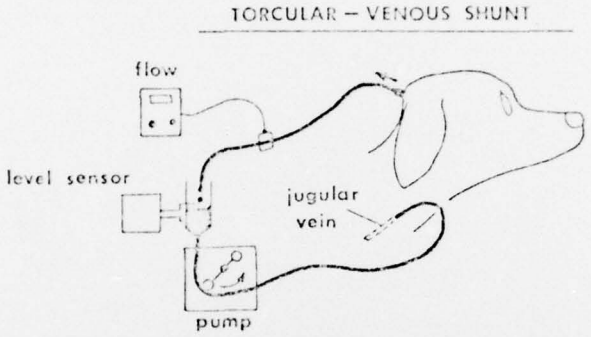


Figure 12. The cerebral blood flow preparation for the dog. An outflow cannula is inserted into the confluens of the cerebral venous sinuses through a hole drilled in the cranial midline below the external occipital protuberance. The lateral cerebral venous sinuses are occluded by injection of a wax with a melting point of 50°C. The cerebral venous outflow passes through an extracorporeal flow probe (Carolina Medical) and then into a revervoir with a level-sensing device which controls the return pump. Cerebral venous pressure is controlled by adjusting the hydrostatic level of the outflow tube opening with respect to the interauditory zero plane. Blood is returned through the external jugular vein.

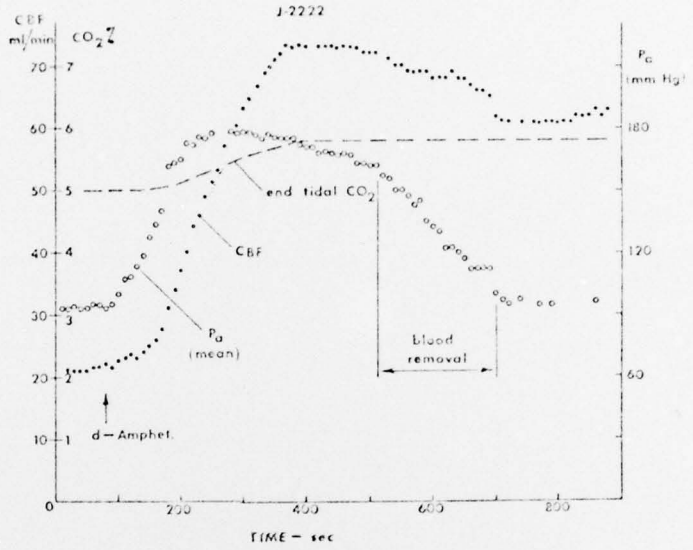


Figure 13. Effects of d-amphetamine (3 mg/kg i.v.) on cerebral blood flow and arterial pressure in the anesthetized dog (Na Pentobarbital 35 mg/kg). Several important points are demonstrated in this figure: note that the arterial pressure is in advance of increases in cerebral blood flow (CBF) and end-tidal CO_2 changes. We suggest two possible mechanisms for the amphetamine effect. One possibility is that metabolic changes in the tissue, stimulated by the drug, establish conditions that require increased blood flow. The other is that the drug exerts a direct effect on the cerebral vasculature, acting as a vasoactive hormone, or stimulating the release of catecholamines from nerve terminals in the vessels themselves. In order to clearly separate the increased blood flow effects from the pressure effects (since there is no need to postulate an active process when there are large increases in pressure that may override the autoregulatory nature of the cerebral vascular bed) sufficient blood was removed (150 cc) to bring arterial pressure back to the control level. CBF remained elevated in spite of this, showing something that was not known before - namely that amphetamine has a direct effect on CBF. Ventilation was fixed by the respirator, and the increased end-tidal CO_2 from 5 - 6% represents increased metabolic production of CO_2 by the dog.

Anesthetized Dog Studies:

Ventriculo-Cisternal Perfusion. Using the preparation depicted in Fig. 14, we have found that d-amphetamine decreases the permeability of the blood-brain barrier to chloride, acutely, after the drug (Fig. 15). These decreases in permeability to chloride may be related to the metabolic effects of d-amphetamine, since similar changes are observed following ventilation with 10% CO_2. So long as the chloride permeability is decreased, we feel that this indicates the integrity of the blood-brain barrier during the period immediately following the drug. There is good agreement between the dilution measurements of CSF production and the rates estimated volumetrically in these experiments.

Peripheral Circulatory Studies. Using the right-heart bypass preparation in Fig. 16, we found that peripheral dopamine release is fairly similar in effects to the administration of d-amphetamine observed in the other experiments described previously. Dopamine infusion resulted in significantly increased arterial pressure, pulmonary artery pressure (not shown), mean systemic pressure and venous return (or cardiac output, since the two are equal in the steady state). It is clear that dopamine acts peripherally to increase cardiac output, increase venous tone, and mobilize blood pooled in the splanchnic bed. In future experiments we will infuse other catecholamines, and then administer the drugs under investigation. Blocking drugs were not used in these first three experiments with dopamine, but we will incorporate them in the future experiments.

DISCUSSION

Two findings related to the d-amphetamine response summarize the most important aspects of the work in this report: First, there is a clear difference between the d- and l-isomers in their peripheral and central effects at the same dosage level. This is evidenced by the circulatory variables, and by the pronounced elevation of CNS levels of MHPG by the d-isomer. Although we did not present data for MHPG in regard to the l-isomer, the calculations which are currently underway indicate a considerably diminished response by comparison.

The second finding, that d-amphetamine exerts a direct effect on CBF, is important because it demonstrates the value of CBF as an index of brain metabolic activity and explains the observed increases in CSF production rates after d-amphetamine. This is strongly supportive of the hypothesis that the observed increases in MHPG do in fact reflect activation of specific neuronal populations in the CNS, and further that there are several indirect ways in which to measure the degree of activation, i.e., CBF and CSF production rates. This information will be most useful inthe interpretation of data from the chronic primate studies, and serves to validate the present series of experiments. We were impressed with the magnitude of the change in CSF MHPG levels, and the close correlation of this with similar changes in arterial pressure, cerebral blood flow and CSF production rates.

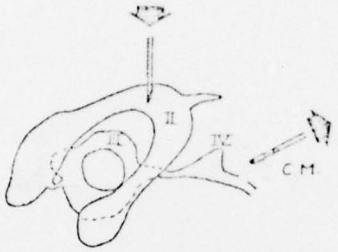


Figure 14. The ventriculo-cisternal perfusion technique in the dog. The inflow needle is placed in the genu of the lateral ventricle (II) near the posterior horn. The outflow catheter is placed in the cisterna magna (C.M.). At the termination of the perfusion, Trypan blue dye is perfused through the system, and the regions actually perfused (shaded area), and catheter placements, visually confirmed at autopsy. Anterior is to left.

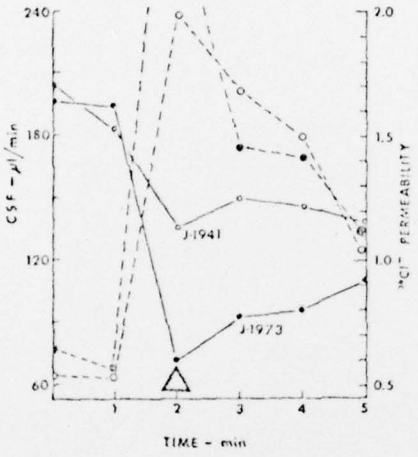


Figure 15. CSF production rates and chloride permeability of the blood-brain barrier after d-amphetamine, 3 mg/kg i.v. (triangle), in anesthetized dogs (Na pentobarbital, 35 mg/kg). These data reflect the transient response to the drug. Chloride (solid line) was moving from CSF to blood, and CSF production (dashed line) was estimated from the ^{14}C-inulin dilution method in the ventriculo-cisternal perfusion system. The permeability to chloride ($^{36}Cl^-$) is inversely related to the secretion of CSF, and brain blood flow from previous figs.

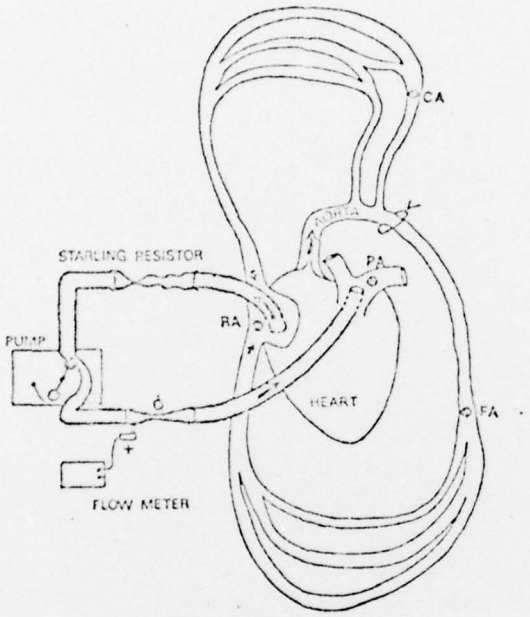


Figure 16. The right-heart bypass preparation. Block dots represent pressure measurements. RA-right atrium; PA-pulmonary artery; CA-carotid artery; and FA- femoral artery. The Starling resistor was manually raised or lowered to keep P_{RA} constant.

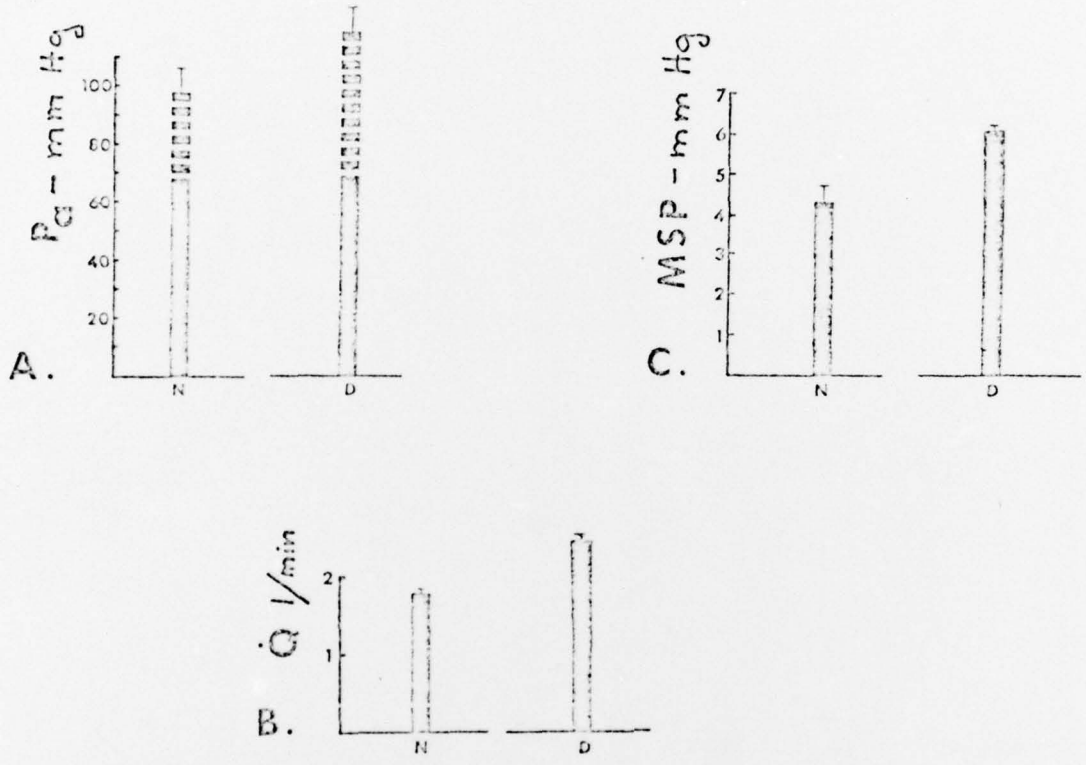


Figure 17. Effects of peripheral dopamine release on the circulation.
A. Arterial pressure. The systolic pressure is represented by the broken line and diastolic by the solid.
B. Venous return (cardiac output). There is a significant increase in venous return with dopamine.
C. Mean systemic pressure (MSP). Dopamine increases central venous pressure, presumably by constricting the splanchnic bed of the circulation. This is the mechanism for the increased venous return.
Vertical bars represent the 95% confidence limits for the mean values from 3 separate experiments.

Possible Metabolic Action of d-Amphetamine on CBF.

If d-amphetamine does not act to release vasoactive hormones from nerve endings in the cerebral vessel walls, then it may act through an indirect metabolic mechanism. For example, if the increased neuronal activity were accompanied by a simultaneous increase in the demand for oxygen and a buildup of acid-metabolites from cellular respiration, then there would be selective, local changes in cerebral blood flow to these tissue regions. We have observed rather profound increases in cerebral blood flow, and these increases are remarkably similar to those produced by ventilation with gas mixtures containing 10% CO_2. We would expect the CO_2 effect to be a more generalized phenomenon, with more even increases in flow to different neural regions. For that matter it is not even known just how CO_2 can do this. If amphetamine acts to release norepinephrine from nerve endings in cerebral vessels, this release could either be direct or triggered by the metabolic production of CO_2 in the cerebral tissues. We feel that it is important to distinguish between these alternatives in order to describe the total drug effect.

SUMMARY and CONCLUSIONS

The following experiments were conducted on dogs acutely to study the effects of d- and l-amphetamine on CSF neurochemical and cardiovascular parameters:

d-amphetamine (3 mg/kg)	J-2511, J-2715, J-2716, J-2753, J-2832, J-2949
l-amphetamine (3 mg/kg)	J-2712, J-2856, J-2912
Control	J-2764, J-2787, J-2888 J-2897

The following experiments were conducted using the right heart bypass to study the peripheral circulation:

J-1887
J-2170
J-2176

The following experiments were conducted on dogs acutely to determine Chloride permeability of the blood-brain barrier after d-amphetamine:

J-1941
J-1973

The The following experiments were conducted on dogs to determine effects of amphetamines on CBF:

We conclude that the d-amphetamine isomer is significantly more potent than the l-isomer in a number of different physiologic systems. The differences appear to be both qualitative and quantitative. The next phase of our study of amphetamines will be to determine dose-response relationships, and to continue the studies in the intact primate model and the acute dog model.

Literature Cited

Dring, L.G., R.L. Smith and R.T. Williams. The fate of amphetamine in man and other mammals. J. Pharm. Pharmacol. 18:402-404, 1966.

Rapela, C.E. and H.D. Green. Autoregulation of Canine Cerebral Blood Flow. Circ. Res. 15:205-211, 1964.

ANNUAL REPORT

Distribution List:	No. Copies
HQDA (SGRD-IDS) Washington, D.C. 20314	4
Defense Documentation Center (DDC) ATTN:DDCIR Cameron Station Alexandria, Virginia 22314	12
Commander U.S. Army Combat Development Command Medical Service Agency Brooke Army Medical Center Fort Sam Houston, Texas 78234	1

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of Environmental Medicine
The Johns Hopkins University
Baltimore, Maryland 21205

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3. REPORT TITLE
"Biochemical Analysis of Cerebrospinal Fluid in Response to Drug Administration"

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Annual Report, June 1, 1973 - May 31, 1974.

5. AUTHOR(S) (First name, middle initial, last name)
Barry Burns, Ph.D.

6. REPORT DATE
January 7, 1974

7a. TOTAL NO. OF PAGES
40

7b. NO. OF REFS
2

8a. CONTRACT OR GRANT NO.
Contract DADA17-73C3129

b. PROJECT NO.

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
Number 03

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT
DISTRIBUTION UNLIMITED

11. SUPPLEMENTARY NOTES
It was found

12. SPONSORING MILITARY ACTIVITY
U.S. Army Medical R&D Command
Washington, D.C. 20314

13. ABSTRACT

Using dogs, we found that d-amphetamine (3 mg/kg i.v.) produces a threefold increase in CSF levels of the norepinephrine metabolite MHPG. L-amphetamine at the same dose level produced either no change in MHPG or had a relatively smaller effect. Cerebral blood flow increased threefold with d-amphetamine at 3 mg/kg in dogs, and only slightly after the l-isomer at the same dose. Arterial pressure doubled after d-amphetamine (3 mg/kg) and only increased 20-30% after the l-isomer at the same dose. We observed significant increases in the CSF production rate after the d-isomer, and little or no change after the l-isomer. A close correlation between arterial pressure, brain blood flow and CSF production rate was observed for d-amphetamine. The d-isomer also resulted in pronounced myocardial effects at 3 mg/kg, whereas the l-isomer did not. The myocardial effects consisted of multiple ventricular foci on the ECG, and were not observed with the l-isomer. Some PVC's were observed with the l-isomer. The effect of the d-isomer on cerebral blood flow was shown to be due to a direct action and was not related to the presser effects. The d-isomer at 3 mg/kg also decreased the permeability of the blood-brain barrier to Chloride during ventriculo-cisternal perfusions in the dogs.

In Primate studies the authors have shown diurnal variations in the CSF production rate and CSF levels of MHPG.

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Drug Addiction						
Amphetamines						
Circulation						
Brain Blood Flow						
Metabolism						
Neurotransmitters						
Primates						
M. mulatta						
Blood Pressure						
Cerebrospinal Fluid						