PUPIL RESPONSES TO INTRAVENOUS HEROIN (DIAMORPHINE) IN DEPENDENT AND NON-DEPENDENT HUMANS

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1 Intravenous heroin was administered to volunteers, in doses of 2.5 and 5 mg to non-dependent subjects and doses of $\frac{1}{3}$, $\frac{1}{2}$ and $\frac{2}{3}$ of their prescribed daily dose of opiates to dependent subjects, and pupillary responses measured before and three times during the 2 h after injection.

2 Tolerance to the miotic effects of heroin in the dependent subjects was demonstrated—larger doses of heroin were needed to produce the same pupil response in dependent subjects than in non-dependent subjects and the duration of action was shorter in the former group.

3 The effect of concurrent oral methadone medication on pupil response to heroin was demonstrated. Subjects prescribed both methadone and heroin showed smaller control pupil diameters and a reduced dose effect to heroin than did subjects prescribed heroin alone.

Introduction

One of the major actions of the opium alkaloids in man is constriction of the pupils. Pin-point pupils are pathognomic of morphine poisoning and one of the signs used in the recognition of opiate dependence is miosis. The pupil diameter is easy to measure (Jasinski & Martin, 1967; Jones, Martin, Isbell and Fraser, 1962) and has been used in many studies of the effects of opiates and opiate-like drugs (e.g. Martin & Fraser, 1961; Fraser & Isbell, 1952). However, as most of this work has been carried out on post-addict volunteers with single doses of subcutaneous or intramuscular opiates, very little is known about the pupil responses to intravenous opiates, in particular heroin (diamorphine) in the dependent subject receiving maintenance heroin treatment. Also the effect of concurrent medication with methadone on the pupil response to heroin is not known.

The question of tolerance to the miotic effect of heroin has also remained unresolved. The fact that the high dose heroin user continues to have constricted pupils has led to the idea that the pupil response to opiates does not show tolerance. This view is reinforced in the literature from the ambiguous '... tolerance to the miotic effect of morphine is not prominent and the morphine addict continues to have constricted pupils' (Goodman & Gilman, 1970) to the incorrect 'Tolerance to the miotic effect (of morphine) is not developed and consequently constricted pupils are observed in the morphine addict' (Reynolds & Randall, 1957). The opiate dependent person certainly

exhibits constricted pupils but he is typically using many times the dose required to produce the same constriction in the non-dependent person. The repeated use of the drug results in a larger dose of drug being required to produce the miosis i.e. tolerance. Some tolerance to the pupillary response to heroin does occur as has been shown by correlating plasma morphine concentrations and pupil diameters before and after heroin injection in dependent and non-dependent subjects (Tress, El-Sobky, Aherne & Piall, 1978). This work was part of a larger study of the pharmacological effects of heroin. The work reported here, the purpose of which was to investigate the effects of intravenous heroin on pupil diameter in non-dependent and dependent subjects with particular reference to tolerance effects and the effect of concurrent methadone medication, was a part of this study.

Methods

The non-dependent subjects were five male healthy volunteers from among the clinical and scientific staff involved in the project. The doses given, 2.5 mg and 5 mg heroin, were chosen from the lower end of the clinically used range and were separated from each other and the saline placebo by at least 1 week to minimize any risk of initiating dependence in these subjects. In addition each volunteer was interviewed by a consultant psychiatrist prior to testing and was

only allowed to go ahead with the experiment if, in the opinion of the psychiatrist, it was prudent for him to do so. The subject then signed a consent form. If, after a dose, a volunteer expressed any doubts or concern about his reaction to the injection he was excluded from further tests. These procedures (and those described below for the dependent subjects) had the approval of the Ethical Review Committees of both Friern Hospital and University College Hospital.

The dependent subjects were volunteers from London Drug Dependency Clinics. Only patients being prescribed heroin or heroin plus methadone (where the methadone consumption in mg did not exceed half of the total daily prescription in mg) were considered for the study. Other requirements were that the patient should have been stabilized on the same prescription for the previous 3 months and that the physician in charge should agree to him participating in the study. Although over 1000 patients were attending these clinics only 60 fulfilled these requirements. When they were approached 30 of them agreed to be subjects and booked a date to come for testing. Only 23 actually came and 3 of these were found to be too ill to test on arrival. Of the remaining 20, one subject was too disruptive to record any meaningful data and one subject was discovered to have been buying amphetamines during her stay so testing was discontinued. In all 18 dependent volunteers (12 male and 6 female) with prescribed daily doses of opiates (heroin and heroin plus methadone) from 30–340 mg were tested. The procedures and purposes of the experiment were again explained to the subjects in the presence of a witness from the Medical Staff and the subjects were asked to sign a consent form. This form was countersigned by one experimenter and by the witness whose function was to ensure that the subject had given informed consent to the procedure.

The heroin dependent subjects were divided into two groups—a high dose and a low dose group. The dividing line between the two groups was set at a daily prescription of 65 mg opiate per day as this was the median dose of heroin being prescribed in the clinics at the start of the study. Patients receiving 65 mg or less per day were assigned to the low dose group of subjects. Patients receiving more than 65 mg per day were assigned to the high dose group. Six of the dependent subjects tested were receiving methadone as

part of their prescription. Three of these subjects were in the high dose and three in the low dose group.

All non-dependent subjects came to the laboratory in the morning for testing and went or were taken home in the afternoon. The dependent subjects were admitted to the Unit for 5 days during which time they stayed in a bed-sitting room. The first 2 days were to allow drugs other than the prescription to wash out prior to testing. To ascertain that other drugs were not being used daily urine samples were taken and analysed for presence of barbiturates, amphetamines and opiates. Testing of the dependent subjects took place on the final three days of their stay.

Non-dependent subjects were tested on three separate occasions with different doses of drug, 5 mg and 2.5 mg heroin and a saline placebo in counter-balanced order. Dependent subjects were also tested on three separate occasions but their test dose was based on their daily prescription. Each subject received $\frac{1}{3}$, $\frac{1}{2}$ and $\frac{2}{3}$ of their daily prescription in counterbalanced order and, on the second test day, a saline placebo was administered 40 min before the test dose of drug. As the test dose of drug was administered at approximately 10.30 h all dependent subjects were given a small dose (10 mg for the low dose group and 20 mg for the high dose group) of heroin at 08.00 h to prevent withdrawal symptoms during the experiment. When methadone was a part of the prescription this was taken before 22.00 h the night before the experiment.

Apart from the details of drug dosage, timing and obtaining subjects described above both the dependent and non-dependent subjects were tested in the same way and under the same conditions. Each experiment was started at 09.30 h when the subject was taken into a test chamber—a soundproof cubicle within a larger laboratory. The subject was seated in a reclining chair whilst transducers were attached. Physiological measures, including pupil diameter measures were repeated at intervals of approximately 20 min twice before injection and until 2 h had elapsed since the

Table 1 Analysis of variance table of the effects of time from injection and dose of heroin on pupil diameter responses in all dependent subjects ($n = 16$)

	<i>F ratio</i>	<i>Degrees of freedom</i>	<i>P</i>
Time from injection	33.00	2,30	<0.005
Dose of heroin	7.60	2,30	<0.005
Time by dose interaction	2.54	4,60	<0.05

Table 2 Analysis of variance table of effects of time from injection, dose of heroin and dependent subject group (high dose group $n = 8$, low dose group $n = 8$)

	<i>F ratio</i>	<i>Degrees of freedom</i>	<i>P</i>
Group	1.48	1,14	NS
Time	32.45	2,28	<0.005
Dose	7.77	2,28	<0.005
<i>Interactions</i>			
Time by dose	2.43	4,56	=0.058
Group by time	0.43	2,28	NS
Group by dose	1.43	2,28	NS
Group by time by dose	0.24	4,56	NS

injection, when the subject was allowed to leave the laboratory.

A record of the pupil diameter was made in the constant dim illumination of the test chamber by photographing the subject's eye together with a 10 mm scale using colour reversal film. The camera was effectively fixed-focus with a telephoto lens and bellows preset to standard magnification. The photograph was taken with the aid of an electronic ring flash. The resulting colour transparencies were put in a photographic enlarger and the horizontal diameter of the pupil measured using the photographed scale. Blood samples were also taken and plasma was assayed using a radioimmunoassay for morphine (Aherne, Piall, Robinson, Morris & Marks, 1975). The results of the plasma concentration measures and their relationship to pupil measures in these experiments are reported elsewhere (Tress, Aherne, El-Sobky, Piall & Marks, 1977; Tress *et al.*, 1978).

The other measures comprised heart rate, respiration rate, and body temperature which were continuously monitored on a pen recorder. The results from these measures and from blood pressure measures will be reported in a future paper (Tress and El-Sobky, in preparation).

Results

One non-dependent subject withdrew himself from the study after completing one session with a dose of 2.5 mg heroin. He reported that his euphoric response to heroin had disturbed him and that he preferred not to take heroin again. The results from the non-dependent subjects are therefore based on four complete sets of data. One dependent subject was only able to complete two sessions due to illness and the results of one session of a second subject were lost due to apparatus failure. Thus there are sixteen complete

sets of data for dependent subjects, the results for the different sessions being based on data from the following numbers of subjects: $\frac{1}{4}$ dose, $n = 18$; $\frac{1}{2}$ dose, $n = 17$; $\frac{3}{4}$ dose, $n = 17$.

Dependent subjects

The results of the pupil diameter measures are displayed in Figure 1. The mean pupil diameters before and at 15 min, 1 h and 2 h after all doses heroin are shown for the dependent group in Figure 1a. Mean pupil diameter is also shown at 15 min following placebo injection. The results for each dose of drug for the dependent subjects were analysed separately using an analysis of variance and in each case a highly significant time effect was found ($\frac{1}{4}$ dose: $F = 43.0$, $\frac{1}{2}$ dose: $F = 63.3$, $\frac{3}{4}$ dose: $F = 47.8$, d.f. = 3, 51, $P < 0.005$). Dunnett's test (Edwards, 1972) was then carried out to compare pupil diameters at 15 min, 1 h and 2 h post drug with the pre drug pupil diameters. After all doses of heroin and at all three times after injection the pupil diameters were significantly reduced in size compared to the control pupil diameters (Dunnett's test, t -values ranged from 7.12 to 42.5, d.f. = 51, all significant at beyond the 1% level).

In order to look at the effects of time from injection and dose of heroin on pupil diameters in the dependent subjects pupil diameters at all doses and times of injection were corrected for control pupil diameter by taking post drug from control diameter and performing an analysis of variance (see Table 1). The results of this analysis were that there were significant effects of both time and heroin dose on pupil diameters and also an interaction between time and dose.

The question of differences between the high dose dependent group and the low dose dependent group was answered by performing another analysis of variance on the data split into two groups of eight subjects (high and low dose groups), (Table 2). Again both time and dose were significant although the time/dose interaction just failed to reach significance. However there was no significant difference between the high and low dose groups nor was there any interaction between subjects and time or dose.

Table 3 Analysis of variance table of effects of time from injection, dose of heroin and dependent subject group (those dependents prescribed heroin plus methadone $n = 6$, those prescribed heroin alone $n = 10$)

	<i>F ratio</i>	<i>Degrees of freedom</i>	<i>P</i>
Group	1.31	1, 14	NS
Time	33.46	2, 28	< 0.005
Dose	5.83	2, 28	< 0.005
<i>Interactions</i>			
Time by dose	1.91	4, 56	NS
Group by time	0.73	2, 28	NS
Group by dose	3.38	2, 28	< 0.05
Group by time by dose	2.06	4, 56	NS

Table 4 Analysis of variance table of the effects of time from injection and dose of heroin on pupil diameter responses in the non-dependent subjects ($n = 4$)

	<i>F ratio</i>	<i>Degrees of freedom</i>	<i>P</i>
Time	3.18	2, 6	NS
Dose	7.94	1, 3	< 0.05 (one-tail test)
Time by dose interaction	2.00	2, 6	NS

Figure 1 (a) Mean pupil diameter before and after intravenous saline placebo (▲) and heroin ($\frac{1}{8}$ (■), $\frac{1}{4}$ (○) and $\frac{1}{2}$ (●) of total daily opiate prescription) in all dependent subjects. Only one post-placebo pupil diameter measure was made as dependent subjects were unable to tolerate an inactive injection for more than 40 min. (b) Mean pupil diameters before and after intravenous saline placebo (▲) and 2.5 (■) and 5 mg (●) heroin in the non-dependent subjects.

The effect of methadone in addition to the heroin on pupil diameter was tested by performing a further analysis of variance with all the dependent subjects divided into those subjects prescribed heroin alone ($n=10$) and those prescribed heroin plus methadone ($n=6$). The means used in this analysis are displayed

in Table 2. Again both time and dose were significant but the time/dose interaction failed to reach significance. There was no significant difference between subject groups but there was a significant interaction between subjects and dose though the subject/time interaction was not significant. Comparison of the mean pre-drug pupil diameters showed that the methadone plus heroin users had significantly smaller pupils than the heroin alone users ($t=2.06$, d.f. = 14, $P<0.05$ one tailed).

During the period of testing (the final 3 days of the subject's stay) no dependent subject was found to have present in their urine any other drug other than the drugs they were prescribed.

Non-dependent subjects

The mean pupil diameters for the non-dependent subjects before and at 15 min, 1 h and 2 h after heroin and placebo are shown in Figure 1b. The results for the two doses of drug and placebo were analysed separately using an analysis of variance. A highly significant time effect was found for both doses of drug (2.5 mg heroin: $F=7.18$, d.f. = 3,9, $P=0.009$, 5 mg heroin: $F=22.08$, d.f. = 3,9, $P=0.0002$) but placebo had no significant effect. Dunnett's test was then carried out on the pupil diameter measures post drug with the control (pre-drug) measures. Pupil diameters following the 5 mg and 2.5 mg doses were all significantly different from the control level (Dunnett's test, t -values ranged from 4.02, to 6.99, d.f. = 9, $P<0.01$ except for 2 h post - 2.5 mg, $t=3.36$, $P<0.05$).

As for the dependent subjects the results for the non-dependent subjects were corrected for control pupil diameter by taking post-drug levels from the control level. An analysis of variance was carried out on these measures (Table 4) and it was found that there was a significant dose effect but unlike the dependent subjects, no significant time effect or time/dose interaction.

Discussion

The results of this study indicate that the miotic effect of intravenous heroin is related to both time and dose and that the responses of dependent and non-dependent subjects differ. Although the test doses of heroin were 2.5 mg and 5 mg for the non-dependent subjects and in the range of 5 to 170 mg for the dependent subjects the initial (15 min) change in pupil diameter to the dose of heroin was in the same range (means of 2.13 and 2.94 mm for 2.5 and 5.0 mg in the non-dependent subjects and 2.11, 3.20 and 3.09 for $\frac{1}{8}$, $\frac{1}{4}$ and $\frac{1}{2}$ dose in the dependent subjects). The difference between the groups lay in the response over time. At all three doses of drug the dependent subjects showed

some recovery of pupil diameter at 2 h after injection and this was reflected in the significant time effect on the analysis of variance. However the non-dependent subjects showed very little recovery in 2 h and there was no significant time effect.

These results clearly demonstrate tolerance to the miotic effect of heroin in two ways. First, to produce similar changes in pupil diameter a larger dose of heroin was required in the dependent group than in the non-dependent group. Second, the duration of action of heroin is shorter in the dependent subjects than in the non-dependent subjects.

More evidence of tolerance came from the comparison of the high-dose group of dependent subjects with the low-dose group. In this case there was no difference between the groups and no interaction between group and time from heroin injection or dose of heroin. This means that, although the high dose group were receiving much higher test doses of heroin than the low dose group, the two groups showed similar pupil responses. That is, a given fraction of the daily opiate prescription will produce the same miotic response regardless of the size of the daily prescription. This suggests that tolerance to the miotic response to heroin increases with the increase in tolerance to its euphoric effect, or, at least, with the increasing amount of heroin required to prevent the onset of withdrawal symptoms.

The effects of concurrent methadone medication on the pupil response to heroin are interesting. Although the last methadone dose was taken before 22.00 h the previous night the heroin plus methadone users

showed smaller pupil size prior to test injection than the heroin-only users. The more interesting result was the interaction between subjects and dose of heroin. Reference to Table 2 shows that the difference between the heroin group and the heroin plus methadone group was in their responses to the different doses of heroin. The heroin group show big differences between the $\frac{1}{4}$ prescription and the $\frac{1}{2}$ and $\frac{3}{4}$ doses whereas the heroin plus methadone group showed the same response for each dose of drug. These results confirm that the prior medication with methadone was still affecting both control pupil diameter and pupillary response to heroin 12 h or more after the methadone dose. Although there still appears to be some disagreement about the plasma half-life of methadone it is evident from the work of Verebely, Volavka, Mulé & Resnick (1975), who showed a plasma half-life of 22 h with chronic administration of methadone, that there would have been a considerable amount of methadone still present in the plasma of these subjects when they were tested. It is to be presumed that the presence of methadone in the plasma resulted in the lower control pupil diameters in the group of methadone-using subjects. Also the changed response to heroin in this group may have been due to the methadone partially blocking the effect of the heroin.

We wish to acknowledge the financial assistance of the Medical Research Council (Contract A806/4). We also thank Dr M. Mitcheson and Dr J. Willis for their help in obtaining subjects for this study.

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(Received April 11, 1978)